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(54) Title: NOVEL PROTEINS AND NUCLEIC ACIDS ENCODING SAME

(57) Abstract: Disclosed herein are nucleic acid sequences that encode G-coupled protein-receptor related polypeptides. Also disclosed are polypeptides encoded by these nucleic acid sequences, and antibodies, which immunospecifically-bind to the polypeptide, as well as derivatives, variants, mutants, or fragments of the aforementioned polypeptide, polynucleotide, or antibody. The invention further discloses therapeutic, diagnostic and research methods for diagnosis, treatment, and prevention of disorders involving any one of these novel human nucleic acids and proteins.

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NOVEL PROTEINS AND NUCLEIC ACIDS ENCODING SAME

BACKGROUND OF THE INVENTION

The invention generally relates to nucleic acids and polypeptides. More particularly, the invention relates to nucleic acids encoding novel G-protein coupled receptor (GPCR) polypeptides, as well as vectors, host cells, antibodies, and recombinant methods for producing these nucleic acids and polypeptides.

SUMMARY OF THE INVENTION

The invention is based in part upon the discovery of nucleic acid sequences encoding novel polypeptides. The novel nucleic acids and polypeptides are referred to herein as GPCRX, or GPCR1, GPCR2, GPCR3, GPCR4, and GPCR5 nucleic acids and polypeptides. These nucleic acids and polypeptides, as well as derivatives, homologs, analogs and fragments thereof, will hereinafter be collectively designated as "GPCRX" nucleic acid or polypeptide sequences.

In one aspect, the invention provides an isolated GPCRX nucleic acid molecule encoding a GPCRX polypeptide that includes a nucleic acid sequence that has identity to the nucleic acids disclosed in SEQ ID NOS:1, 3, 5, 6, 8, 9, 10, 12, 14, and 16. In some embodiments, the GPCRX nucleic acid molecule will hybridize under stringent conditions to a nucleic acid sequence complementary to a nucleic acid molecule that includes a protein-coding sequence of a GPCRX nucleic acid sequence. The invention also includes an isolated nucleic acid that encodes a GPCRX polypeptide, or a fragment, homolog, analog or derivative thereof. For example, the nucleic acid can encode a polypeptide at least 80% identical to a polypeptide comprising the amino acid sequences of SEQ ID NOS:2, 4, 7, 11, 13, 15, and 17. The nucleic acid can be, for example, a genomic DNA fragment or a cDNA molecule that includes the nucleic acid sequence of any of SEQ ID NOS:1, 3, 5, 6, 8, 9, 10, 12, 14, and 16.

Also included in the invention is an oligonucleotide, *e.g.*, an oligonucleotide which includes at least 6 contiguous nucleotides of a GPCRX nucleic acid (*e.g.*, SEQ ID NOS:1, 3, 5, 6, 8, 9, 10, 12, 14, and 16) or a complement of said oligonucleotide.

Also included in the invention are substantially purified GPCRX polypeptides (SEQ ID NOS:2, 4, 7, 11, 13, 15, and 17). In certain embodiments, the GPCRX polypeptides include an amino acid sequence that is substantially identical to the amino acid sequence of a human GPCRX polypeptide.

The invention also features antibodies that immunoselectively-binds to GPCRX polypeptides, or fragments, homologs, analogs or derivatives thereof.

In another aspect, the invention includes pharmaceutical compositions that include therapeutically- or prophylactically-effective amounts of a therapeutic and a pharmaceutically-acceptable carrier. The therapeutic can be, *e.g.*, a GPCRX nucleic acid, a GPCRX polypeptide, or an antibody specific for a GPCRX polypeptide. In a further aspect, the invention includes, in one or more containers, a therapeutically- or prophylactically-effective amount of this pharmaceutical composition.

In a further aspect, the invention includes a method of producing a polypeptide by culturing a cell that includes a GPCRX nucleic acid, under conditions allowing for expression of the GPCRX polypeptide encoded by the DNA. If desired, the GPCRX polypeptide can then be recovered.

In another aspect, the invention includes a method of detecting the presence of a GPCRX polypeptide in a sample. In the method, a sample is contacted with a compound that selectively binds to the polypeptide under conditions allowing for formation of a complex between the polypeptide and the compound. The complex is detected, if present, thereby identifying the GPCRX polypeptide within the sample.

The invention also includes methods to identify specific cell or tissue types based on their expression of a GPCRX.

Also included in the invention is a method of detecting the presence of a GPCRX nucleic acid molecule in a sample by contacting the sample with a GPCRX nucleic acid probe or primer, and detecting whether the nucleic acid probe or primer bound to a GPCRX nucleic acid molecule in the sample.

In a further aspect, the invention provides a method for modulating the activity of a GPCRX polypeptide by contacting a cell sample that includes the GPCRX polypeptide with a compound that binds to the GPCRX polypeptide in an amount sufficient to modulate the activity of said polypeptide. The compound can be, *e.g.*, a small molecule, such as a nucleic acid, peptide, polypeptide, peptidomimetic, carbohydrate, lipid or other organic (carbon containing) or inorganic molecule, as further described herein.

Also within the scope of the invention is the use of a therapeutic in the manufacture of a medicament for treating or preventing disorders or syndromes including, *e.g.*, diabetes, metabolic disturbances associated with obesity, the metabolic syndrome X, anorexia, wasting disorders associated with chronic diseases, metabolic disorders, diabetes, obesity, infectious disease, anorexia, cancer-associated cachexia, cancer, neurodegenerative disorders,

Alzheimer's Disease, Parkinson's Disorder, immune disorders, and hematopoietic disorders, or other disorders related to cell signal processing and metabolic pathway modulation. The therapeutic can be, *e.g.*, a GPCRX nucleic acid, a GPCRX polypeptide, or a GPCRX-specific antibody, or biologically-active derivatives or fragments thereof.

For example, the compositions of the present invention will have efficacy for treatment of patients suffering from: developmental diseases, MHCII and III diseases (immune diseases), taste and scent detectability Disorders, Burkitt's lymphoma, Corticoneurogenic disease, Signal Transduction pathway disorders, Retinal diseases including those involving photoreception, Cell Growth rate disorders; Cell Shape disorders, Feeding disorders; control of feeding; potential obesity due to over-eating; potential disorders due to starvation (lack of appetite), noninsulin-dependent diabetes mellitus (NIDDM1), bacterial, fungal, protozoal and viral infections (particularly infections caused by HIV-1 or HIV-2), pain, cancer (including but not limited to neoplasm; adenocarcinoma; lymphoma; prostate cancer; uterus cancer), anorexia, bulimia, asthma, Parkinson's disease, acute heart failure, hypotension, hypertension, urinary retention, osteoporosis, Crohn's disease; multiple sclerosis; Albright Hereditary Ostoeodystrophy, angina pectoris, myocardial infarction, ulcers, asthma, allergies, benign prostatic hypertrophy, and psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, delirium, dementia, severe mental retardation. Dentatorubro-pallidoluysian atrophy (DRPLA) Hypophosphatemic rickets, autosomal dominant (2) Acrocallosal syndrome and dyskinesias, such as Huntington's disease or Gilles de la Tourette syndrome and/or other pathologies and disorders of the like.

The polypeptides can be used as immunogens to produce antibodies specific for the invention, and as vaccines. They can also be used to screen for potential agonist and antagonist compounds. For example, a cDNA encoding GPCRX may be useful in gene therapy, and GPCRX may be useful when administered to a subject in need thereof. By way of nonlimiting example, the compositions of the present invention will have efficacy for treatment of patients suffering from bacterial, fungal, protozoal and viral infections (particularly infections caused by HIV-1 or HIV-2), pain, cancer (including but not limited to Neoplasm; adenocarcinoma; lymphoma; prostate cancer; uterus cancer), anorexia, bulimia, asthma, Parkinson's disease, acute heart failure, hypotension, hypertension, urinary retention, osteoporosis, Crohn's disease; multiple sclerosis; and Treatment of Albright Hereditary Ostoeodystrophy, angina pectoris, myocardial infarction, ulcers, asthma, allergies, benign prostatic hypertrophy, and psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, delirium, dementia, severe mental retardation and

dyskinesias, such as Huntington's disease or Gilles de la Tourette syndrome and/or other pathologies and disorders.

The invention further includes a method for screening for a modulator of disorders or syndromes including, *e.g.*, diabetes, metabolic disturbances associated with obesity, the metabolic syndrome X, anorexia, wasting disorders associated with chronic diseases, metabolic disorders, diabetes, obesity, infectious disease, anorexia, cancer-associated cachexia, cancer, neurodegenerative disorders, Alzheimer's Disease, Parkinson's Disorder, immune disorders, and hematopoietic disorders or other disorders related to cell signal processing and metabolic pathway modulation. The method includes contacting a test compound with a GPCRX polypeptide and determining if the test compound binds to said GPCRX polypeptide. Binding of the test compound to the GPCRX polypeptide indicates the test compound is a modulator of activity, or of latency or predisposition to the aforementioned disorders or syndromes.

Also within the scope of the invention is a method for screening for a modulator of activity, or of latency or predisposition to an disorders or syndromes including, *e.g.*, diabetes, metabolic disturbances associated with obesity, the metabolic syndrome X, anorexia, wasting disorders associated with chronic diseases, metabolic disorders, diabetes, obesity, infectious disease, anorexia, cancer-associated cachexia, cancer, neurodegenerative disorders, Alzheimer's Disease, Parkinson's Disorder, immune disorders, and hematopoietic disorders or other disorders related to cell signal processing and metabolic pathway modulation by administering a test compound to a test animal at increased risk for the aforementioned disorders or syndromes. The test animal expresses a recombinant polypeptide encoded by a GPCRX nucleic acid. Expression or activity of GPCRX polypeptide is then measured in the test animal, as is expression or activity of the protein in a control animal which recombinantly-expresses GPCRX polypeptide and is not at increased risk for the disorder or syndrome. Next, the expression of GPCRX polypeptide in both the test animal and the control animal is compared. A change in the activity of GPCRX polypeptide in the test animal relative to the control animal indicates the test compound is a modulator of latency of the disorder or syndrome.

In yet another aspect, the invention includes a method for determining the presence of or predisposition to a disease associated with altered levels of a GPCRX polypeptide, a GPCRX nucleic acid, or both, in a subject (*e.g.*, a human subject). The method includes measuring the amount of the GPCRX polypeptide in a test sample from the subject and comparing the amount of the polypeptide in the test sample to the amount of the GPCRX

polypeptide present in a control sample. An alteration in the level of the GPCRX polypeptide in the test sample as compared to the control sample indicates the presence of or predisposition to a disease in the subject. Preferably, the predisposition includes, *e.g.*, diabetes, metabolic disturbances associated with obesity, the metabolic syndrome X, anorexia, wasting disorders associated with chronic diseases, metabolic disorders, diabetes, obesity, infectious disease, anorexia, cancer-associated cachexia, cancer, neurodegenerative disorders, Alzheimer's Disease, Parkinson's Disorder, immune disorders, and hematopoietic disorders. Also, the expression levels of the new polypeptides of the invention can be used in a method to screen for various cancers as well as to determine the stage of cancers.

In a further aspect, the invention includes a method of treating or preventing a pathological condition associated with a disorder in a mammal by administering to the subject a GPCRX polypeptide, a GPCRX nucleic acid, or a GPCRX-specific antibody to a subject (*e.g.*, a human subject), in an amount sufficient to alleviate or prevent the pathological condition. In preferred embodiments, the disorder, includes, *e.g.*, diabetes, metabolic disturbances associated with obesity, the metabolic syndrome X, anorexia, wasting disorders associated with chronic diseases, metabolic disorders, diabetes, obesity, infectious disease, anorexia, cancer-associated cachexia, cancer, neurodegenerative disorders, Alzheimer's Disease, Parkinson's Disorder, immune disorders, and hematopoietic disorders.

In yet another aspect, the invention can be used in a method to identity the cellular receptors and downstream effectors of the invention by any one of a number of techniques commonly employed in the art. These include but are not limited to the two-hybrid system, affinity purification, co-precipitation with antibodies or other specific-interacting molecules.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In the case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

Other features and advantages of the invention will be apparent from the following detailed description and claims.

DETAILED DESCRIPTION OF THE INVENTION

The invention is based, in part, upon the discovery of novel nucleic acid sequences that encode novel polypeptides. The novel nucleic acids and their encoded polypeptides are referred to individually as GPCR1, GPCR2, GPCR3, GPCR4, and GPCR5. The nucleic acids, and their encoded polypeptides, are collectively designated herein as "GPCRX".

The novel GPCRX nucleic acids of the invention include the nucleic acids whose sequences are provided in Tables 1A, 2A, 2D, 2E, 2F, 2G, 2J, 3A, 4A, and 5A, inclusive ("Tables 1A - 5A"), or a fragment, derivative, analog or homolog thereof. The novel GPCRX proteins of the invention include the protein fragments whose sequences are provided in Tables 1B, 2B, 2H, 2K, 3B, 4B, and 5B, inclusive ("Tables 1B - 5B"). The individual GPCRX nucleic acids and proteins are described below. Within the scope of this invention is a method of using these nucleic acids and peptides in the treatment or prevention of a disorder related to cell signaling or metabolic pathway modulation.

G-Protein Coupled Receptor proteins (GPCRs) have been identified as a large family of G protein-coupled receptors in a number of species. These receptors share a seven transmembrane domain structure with many neurotransmitter and hormone receptors, and are likely to underlie the recognition and G-protein-mediated transduction of various signals. Human GPCR generally do not contain introns and belong to four different gene subfamilies, displaying great sequence variability. These genes are dominantly expressed in olfactory epithelium. See, e.g., Ben-Arie et al., Hum. Mol. Genet. 1994 3:229-235; and, Online Mendelian Inheritance in Man (OMIM) entry # 164342 (http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?).

The olfactory receptor (OR) gene family constitutes one of the largest GPCR multigene families and is distributed among many chromosomal sites in the human genome. See Rouquier et al., Hum. Mol. Genet. 7(9):1337-45 (1998); Malnic et al., Cell 96:713-23 (1999). Olfactory receptors constitute the largest family among G protein-coupled receptors, with up to 1000 members expected. See Vanderhaeghen et al., Genomics 39(3):239-46 (1997); Xie et al., Mamm. Genome 11(12):1070-78 (2000); Issel-Tarver et al., Proc. Natl. Acad. Sci. USA 93(20):10897-902 (1996). The recognition of odorants by olfactory receptors is the first stage in odor discrimination. See Krautwurst et al., Cell 95(7):917-26 (1998); Buck et al., Cell 65(1):175-87 (1991). Many ORs share some characteristic sequence motifs and have a central variable region corresponding to a putative ligand binding site. See Issel-Tarver et al., Proc. Natl. Acad. Sci. USA 93:10897-902 (1996).

Other examples of seven membrane spanning proteins that are related to GPCRs are chemoreceptors. See Thomas et al., Gene 178(1-2):1-5 (1996). Chemoreceptors have been identified in taste, olfactory, and male reproductive tissues. See id.; Walensky et al., J. Biol. Chem. 273(16):9378-87 (1998); Parmentier et al., Nature 355(6359):453-55 (1992); Asai et al., Biochem. Biophys. Res. Commun. 221(2):240-47 (1996).

GPCR1

Novel GPCR1 is a G-protein coupled receptor ("GPCR") protein related to human olfactory receptors. A disclosed GPCR1 nucleic acid of 980 nucleotides is shown in Table 1A. The disclosed GPCR1 open reading frame ("ORF") begins at the ATG initiation codon at nucleotides 14-16, shown in bold in Table 1A. The encoded polypeptide is alternatively referred to herein as GPCR1, AC021427_A or as AC021427_E. The disclosed GPCR1 ORF terminates at a TAG codon at nucleotides 947-949. As shown in Table 1A, putative untranslated regions 5' to the start codon and 3' to the stop codon are underlined, and the start and stop codons are in bold letters.

Table 1A. GPCR1 nucleotide sequence (SEQ ID NO:1).

CAGGCGAGAGAAC**ATG**ACAAAAGGCAATCGTACCACAGTGACCGAATTTGTCCTCATGGGATTCAC
AGACCGTCCTGAGCTGCAGCTCCCCCTCTTTGTGGTGTTCCTTGTCATTTATCTCATCACCCTGGT
GGGAAACCTTGGCATGATCCTGCTGATCAGAGCAGACTCGCGGCTCCACACCCCCATGTACTACTT
CCTCAGTCACCTGGCATTCATTGATCTGTGTTACTCATCTTCTATTGGGCCCAAGATGCTGCAAAA
TGTATTGGTGAAGAAAAAAACCATCTCCTTTTCAGGCTGTTTTGCTCAGCTGTACTTCTCCGGTGC
TTTTGCCACTACAGAATGTTTCCTCTTGGCCACAATGCCCTACGACCGCTACGTGGCCATCTGCAA
CCCCCTGATTTACACAGCTATTATGACGCAGCGGGTCTGCAGGGAGTTAGTGATAGGGGTCTATAC
CTATGGCTTCCGAAACTCTGTGATACAGACAGCTCTGACGTTTCAGCTGTCTTTCTGCAACTCCGA
CGTCATCCACCACTTCTACTGTGCTGACCCCCCTCTCCTGGCCCTCTCCTGCTCTGACACCCACAA
CAAAGAAAAGCAGCTCATGATCTTCTCTGCAGTAAATCTCACTGGGTCCCTCCTTACCATCTTCAT
CTCCTACATTTGCATCCTCTTTTCCATTATAAAAATCCAGTCTTCCGAGGGCAAGTGCAGAGCATT
TTCCACCCGTGCCTCCCACCTCACTGTCGTCACCATCTTTTATGGCACACTATTTTTCATGTACCT
GCAGCAACCAAAAGCGGGGAATTCATGGAAGCCAAACAAAGTAGTCTCTGTGTTTTATAGTCTTGT
AATTCCCATGCTTAACCCTCTTATCTATCGCCTGAGAAACACAGAAGTAAAGGATGCCCTGAAAAA
AATGCTAGAGGGCAAAGAGTTA**TAG**TGAGTGAGTTAATGGAACGCAGCATACTGAA

A disclosed encoded GPCR1 protein has 311 amino acid residues, referred to as the GPCR1 protein. The GPCR1 protein was analyzed for signal peptide prediction and cellular localization. SignalP results predict that GPCR1 is cleaved between position 44 and 55 of SEQ ID NO:2, *i.e.*, at the slash in the amino acid sequence NLG-MI. Psort and Hydropathy profiles also predict that GPCR1 contains a signal peptide and is likely to be localized at the plasma membrane (certainty of 0.6000). The disclosed GPCR1 polypeptide sequence is presented in Table 1B using the one-letter amino acid code.

Table 1B. Encoded GPCR1 protein sequence (SEQ ID NO:2).
MTKGNRTTVTEFVLMGFTDRPELQLPLFVVFLVIYLITLVGNLG/MILLIRADSRLHTPMYYFLSH LAFIDLCYSSSIGPKMLQNVLVKKKTISFSGCFAQLYFSGAFATTECFLLATMPYDRYVAICNPLI YTAIMTQRVCRELVIGVYTYGFRNSVIQTALTFQLSFCNSDVIHHFYCADPPLLALSCSDTHNKEK QLMIFSAVNLTGSLLTIFISYICILFSIIKIQSSEGKCRAFSTRASHLTVVTIFYGTLFFMYLQQP KAGNSWKPNKVVSVFYSLVIPMLNPLIYRLRNTEVKDALKKMLEGKEL

GPCR1 was initially identified on chromosome 11 with a TblastN analysis of a proprietary sequence file for a G-protein coupled receptor probe or homolog, which was run against the Genomic Daily Files made available by GenBank. A proprietary software program (GenScan™) was used to further predict the nucleic acid sequence and the selection of exons. The resulting sequences were further modified by means of similarities using BLAST searches. The sequences were then manually corrected for apparent inconsistencies, thereby obtaining the sequences encoding the full-length protein.

A region of the AC021427_A nucleic acid sequence has 471 of 475 bases (99%) identical to a sequence coding for a partial *Homo sapiens* olfactory receptor (OR) mRNA (GENBANK-ID: X89671, SEQ ID NO:20), as shown in Table 1C. See, also, Vanderhaegen et al., Genomics 39: 239-246, 1997.

The disclosed, full-length nucleic acid sequence also has 617 of 937 bases (65%) identical to a *Gallus gallus* cor4 DNA for olfactory receptor 4 (GENBANK-ID: X94744, 936 bp; Score = 1601 (240.2 bits), Expect = 3.1e-66, P = 3.1e-66; Identities = 617/937 (65%), Positives = 617/937 (65%), Strand = Plus / Plus).

The nucleic acid was also found to have 475/620 bases (76%) identical and 475/620 (76%) positives to a *Mus musculus* G-protein coupled receptor mRNA (GENBANK-ID:AF121977) ($E= 3.6e^{-69}$).

In all BLAST alignments herein, the "E-value" or "Expect" value is a numeric indication of the probability that the aligned sequences could have achieved their similarity to the BLAST query sequence by chance alone, within the database that was searched. For example, the probability that the subject ("Sbjct") retrieved from the GPCR1 BLAST analysis, *e.g.*, the *Gallus gallus* cor4 DNA for olfactory receptor 4, matched the Query GPCR1 sequence purely by chance is $3.1x10^{-66}$.

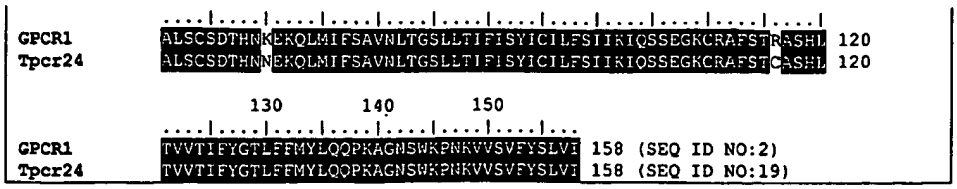
Table 1C. BLASTN of GPCR1 against H. sapiens TPCR24

```
>gi|902325|emb|X89671.1|HSTPCR24P H.sapiens mRNA for TPCR24 protein
          Length = 475 (SEQ ID NO:18)
 Score =  910 bits (459), Expect = 0.0
 Identities = 471/475 (99%)   Strand = Plus / Plus

Query: 388 catctgcaacccccctgatttacacagctattatgacgcagcgggtctgcagggagttagt 447
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 1   catctgcaacccccctgatttacacagctattatgacgcagcgggtctgcagggagttagt 60

Query: 448 gataggggtctatacctatggcttccgaaactctgtgatacagacagctctgacgtttca 507
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 61  gataggggtctatacctatggcttccgaaactctgtgatacagacagctctgacgtttca 120

Query: 508 gctgtctttctgcaactccgacgtcatccaccacttctactgtgctgacccccctctcct 567
           |||||||||||||||||||||||||||||||||||||||||||||||||| |||||||
Sbjct: 121 gctgtctttctgcaactccgacgtcatccaccacttctactgtgctgacccctctctcct 180

Query: 568 ggccctctcctgctctgacacccacaacaaagaaaagcagctcatgatcttctctgcagt 627
           |||||||||||||||||||||||||||| ||||||||||||||||||||||||||||
Sbjct: 181 ggccctctcctgctctgacacccacaacaatgaaaagcagctcatgatcttctctgcagt 240

Query: 628 aaatctcactgggtccctccttaccatcttcatctcctacatttgcatcctcttttccat 687
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 241 aaatctcactgggtccctccttaccatcttcatctcctacatttgcatcctcttttccat 300

Query: 688 tataaaaatccagtcttccgagggcaagtgcagagcattttccacccgtgcctcccacct 747
           |||||||||||||||||||||||||||||||||||||||||| ||||||||||||
Sbjct: 301 tataaaaatccagtcttccgagggcaagtgcagagcattttccacctgtgcctcccacct 360

Query: 748 cactgtcgtcaccatcttttatggcacactatttttcatgtacctgcagcaaccaaaagc 807
           |||||||||||||||||||||||||| ||||||||||||||||||||||||||||||
Sbjct: 361 cactgtcgtcaccatcttttatggcacactgtttttcatgtacctgcagcaaccaaaagc 420

Query: 808 ggggaattcatggaagccaaacaaagtagtctctgtgttttatagtcttgtaatt 862
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 421 ggggaattcatggaagccaaacaaagtagtctctgtgttttatagtcttgtaatt 475 (SEQ
ID NO:18)
```

A BLASTX search was performed against public protein databases. As shown in Table 1D, amino acids 126-283 of the disclosed GPCR1 protein (SEQ ID NO:2) have 98% identity across the 158 amino acid long *Homo sapiens* TPCR24 olfactory receptor (SEQ ID NO:19).

Table 1D. BLAST of GPCR1 against TPCR

```
gi|2135966|pir||S58016 probable olfactory receptor tpcr24 - human
(fragment)
Length = 158 (SEQ ID NO:19)
 Score =  284 bits (726), Expect = 2e-75
 Identities = 155/158 (98%), Positives = 155/158 (98%)

                10        20        30        40        50        60
            ....|....|....|....|....|....|....|....|....|....|....|
GPCR1       ICNPLIYTAIMTQRVCRELVIGVYTYGFRNSVIQTALTFQLSFCNSDVIHHFYCADFSLL 60
Tpcr24      LCNPLIYTAIMTQRVCRELVIGVYTYGFRNSVIQTALTFQLSFCNSDVIHHFYCADFSLM 60

                70        80        90       100       110       120
```



The GPCR1 protein was also found to have 168 of 304 amino acid residues (55%) identical to, and 219 of 304 residues (72%) positive with the 313 amino acid sequence of *Hylobates lar* (common gibbon) olfactory receptor OR93GIB (ptnr: STREMBL-ACC:O77758) (E= 7.0 e^{-86}). The full GPCR1 amino acid also shows 214 of 312 amino acids (68%) identical to and (79%) positive with the 342 amino acid odorant receptor protein from *Mus musculus* (ptnr: STREMBL-ACC:Q9WU91) (E= 3.9 e^{-108}).

Additionally, the GPCR1 protein was also found to have 167 of 306 amino acid residues (54%) identical to, and 228 of 306 residues (74%) positive with the 305 amino acid sequence of *Homo sapiens* olfactory protein OLF-5 (patp: Y83390, PCT publication WO 00/21985)
(E= 6.5 e^{-87}). Patp is a proprietary database that contains sequences published in patents and patent publications. Other BLAST results from Patp include:

Table 1E. Patp alignments of GPCR1

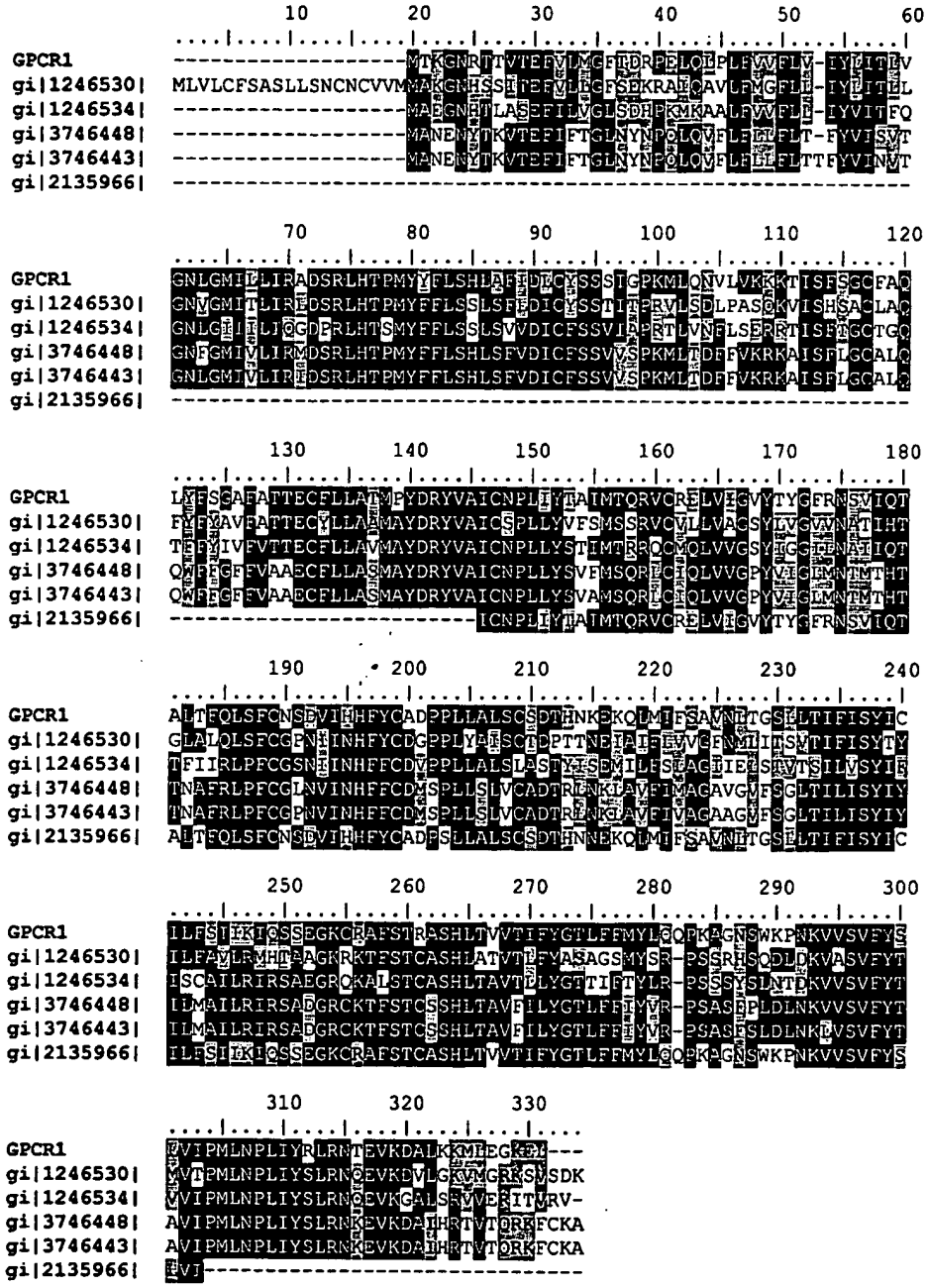
Sequences producing High-scoring Segment Pairs:
Smallest

	Reading Frame	High Score	Sum Prob. P(N)
patp:Y83390 Olfactory receptor protein OLF-5 - Homo sapiens.	+2	876	6.5e-87
patp:Y83387 Olfactory receptor protein OLF-2 - Homo sapiens...	+2	859	4.1e-85
patp:Y83389 Olfactory receptor protein OLF-4 - Homo sapiens...	+2	856	8.5e-85
patp:Y83394 Olfactory receptor protein OLF-9 - Homo sapiens...	+2	838	6.9e-83
patp:Y90877 Human G protein-coupled receptor GTAR11-3 ..	+2	783	4.6e-77
patp:Y90876 Human G protein-coupled receptor GTAR11-2 ..	+2	773	5.3e-76

A ClustalW analysis comparing the protein of the invention with related protein sequences is given in Table 1F, with GPCR1 shown on line X. In the ClustalW alignment of the GPCR1 protein, as well as all other ClustalW analyses herein, the black outlined amino acid residues indicate regions of conserved sequence (*i.e.*, regions that may be required to preserve structural or functional properties), whereas non-highlighted amino acid residues are less conserved and can potentially be mutated to a much broader extent without altering protein structure or function.

Table 1F. ClustalW Analysis of GPCR1

1) (AC021427_A) Novel GPCR, SEQ ID NO:2
2) gi|1246530|emb|CAA64368.1| olfactory receptor 2 [Gallus gallus], SEQ ID NO:20
3) gi|1246534|emb|CAA64370.1| olfactory receptor 4 [Gallus gallus], SEQ ID NO:21
4) gi|3746448|gb|AAC63971.1| olfactory receptor OR93Gib [Hylobates lar], SEQ ID NO:22
5) gi|3746443|gb|AAC63969.1| olfactory receptor OR93Ch [Pan troglodytes], SEQ ID NO:23
6) gi|2135966|pir||S58016 probable olfactory receptor tpcr24 - human (fragment), SEQ ID NO:19



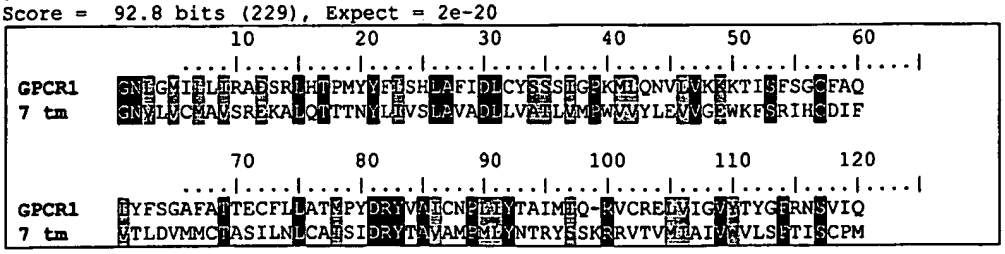
The presence of identifiable domains in GPCR1, as well as all other GPCRX proteins, was determined by searches using software algorithms such as PROSITE, DOMAIN, Blocks,

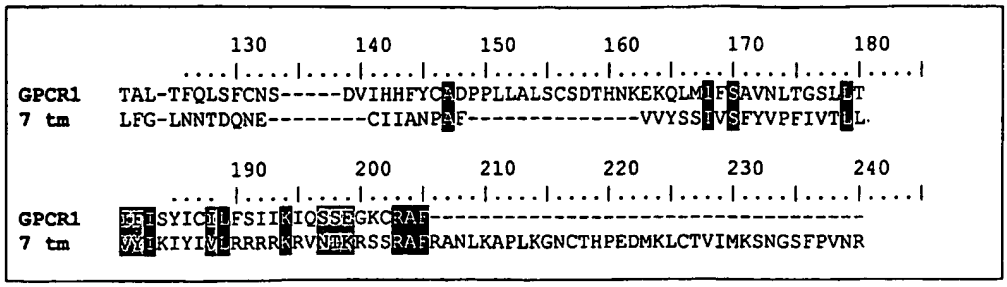
Pfam, ProDomain, and Prints, and then determining the Interpro number by crossing the domain match (or numbers) using the Interpro website (http:www.ebi.ac.uk/ interpro). DOMAIN results, *e.g.*, for GPCR1 as disclosed in Table 1G, were collected from the Conserved Domain Database (CDD) with Reverse Position Specific BLAST analyses. This BLAST analysis software samples domains found in the Smart and Pfam collections. For Table 1G and all successive DOMAIN sequence alignments, fully conserved single residues are indicated by black shading and "strong" semi-conserved residues are indicated by grey shading. The "strong" group of conserved amino acid residues may be any one of the following groups of amino acids: STA, NEQK, NHQK, NDEQ, QHRK, MILV, MILF, HY, FYW.

Table 1G lists the statistics and domain description from DOMAIN analysis results against GPCR1. The region from amino acid residue 41 through 238 (numbered with respect to SEQ ID NO:2) most probably ($E = 2x10^{-20}$) contains a "seven transmembrane receptor (rhodopsin family) fragment" domain, aligned here with residues 1-182 of the 7tm_1 entry (SEQ ID NO:24) of the Pfam database. This indicates that the GPCR1 sequence has properties similar to those of other proteins known to contain this domain as well as to the 7tm_1 domain itself. The 7 transmembrane receptor family includes a number of different proteins, including, for example, serotonin receptors, dopamine receptors, histamine receptors, andrenergic receptors, cannabinoid receptors, angiotensin II receptors, chemokine receptors, opioid receptors, G-protein coupled receptor (GPCR) proteins, olfactory receptors (OR), and the like. Some proteins and the Protein Data Base Ids/gene indexes include, for example: rhodopsin (129209); 5-hydroxytryptamine receptors; (112821, 8488960, 112805, 231454, 1168221, 398971, 112806); G protein-coupled receptors (119130, 543823, 1730143, 132206, 137159, 6136153, 416926, 1169881, 136882, 134079); gustatory receptors (544463, 462208); c-x-c chemokine receptors (416718, 128999, 416802, 548703, 1352335); opsins (129193, 129197, 129203); and olfactory receptor-like proteins (129091, 1171893, 400672, 548417);

Table 1G. DOMAIN results for GPCR1

```
Sbjct: 7 transmembrane receptor (rhodopsin family) fragment (SEQ ID NO:24)
gnl|Pfam|pfam00001;  Length = 377
Score =  92.8 bits (229), Expect = 2e-20
```





Expression information for GPCRX RNA was derived using tissue sources including, but not limited to, proprietary database sources, public EST sources, literature sources, and/or RACE sources, as described in the Examples.

The nucleic acids and proteins of GPCR1 are useful in potential therapeutic applications implicated in various GPCR- or olfactory receptor (OR)-related pathologies and/or disorders. For example, a cDNA encoding the G-protein coupled receptor-like protein may be useful in gene therapy, and the G-protein coupled receptor-like protein may be useful when administered to a subject in need thereof. The novel nucleic acid encoding GPCR1 protein, or fragments thereof, may further be useful in diagnostic applications, wherein the presence or amount of the nucleic acid or the protein are to be assessed. These materials are further useful in the generation of antibodies that bind immunospecifically to the novel substances of the invention for use in therapeutic or diagnostic methods. The GPCRX nucleic acids and proteins are useful in potential diagnostic and therapeutic applications implicated in various diseases and disorders described below and/or other pathologies. For example, the compositions of the present invention will have efficacy for treatment of patients suffering from: cardiomyopathy, atherosclerosis, hypertension, congenital heart defects, aortic stenosis, atrial septal defect (ASD), atrioventricular (A-V) canal defect, ductus arteriosus, pulmonary stenosis, subaortic stenosis, ventricular septal defect (VSD), valve diseases, tuberous sclerosis, scleroderma, obesity, transplantation, adrenoleukodystrophy, congenital adrenal hyperplasia, fertility, hemophilia, hypercoagulation, idiopathic thrombocytopenic purpura, immunodeficiencies, graft versus host disease, bronchial asthma, and other diseases, disorders and conditions of the like. By way of nonlimiting example, the compositions of the present invention will have efficacy for treatment of patients suffering from neoplasm, adenocarcinoma, lymphoma, prostate cancer, uterus cancer, immune response, AIDS, asthma, Crohn's disease, multiple sclerosis, and Albright Hereditary Ostoeodystrophy. Additional GPCR-related diseases and disorders are mentioned throughout the Specification.

Further, the protein similarity information, expression pattern, and map location for GPCR1 suggests that GPCR1 may have important structural and/or physiological functions

characteristic of the GPCR family. Therefore, the nucleic acids and proteins of the invention are useful in potential diagnostic and therapeutic applications and as a research tool. These include serving as a specific or selective nucleic acid or protein diagnostic and/or prognostic marker, wherein the presence or amount of the nucleic acid or the protein are to be assessed, as well as potential therapeutic applications such as the following: (i) a protein therapeutic, (ii) a small molecule drug target, (iii) an antibody target (therapeutic, diagnostic, drug targeting/cytotoxic antibody), (iv) a nucleic acid useful in gene therapy (gene delivery/gene ablation), and (v) a composition promoting tissue regeneration *in vitro* and *in vivo* (vi) biological defense weapon.

These materials are further useful in the generation of antibodies that bind immunospecifically to the novel GPCR1 substances for use in therapeutic or diagnostic methods. These antibodies may be generated according to methods known in the art, using prediction from hydrophobicity charts, as described in the "Anti-GPCRX Antibodies" section below. In one embodiment, a contemplated GPCR1 epitope is from about amino acids170 to 210. In another embodiment, a GPCR1 epitope is from about amino acids230 to 250. In additional embodiments, GPCR1 epitopes are from amino acids260 to 280 and from amino acids290 to 310.

GPCR2

GPCR2 includes a family of six nucleic acids disclosed below. The disclosed nucleic acids encode three GPCR-like proteins, which are each 311 amino acids in length. The three encoded proteins, which have been named GPCR2a, GPCR2b, and GPCR2c differ from each other by one amino acid.

GPCR2a

The disclosed GPCR2a is encoded by four different nucleic acids, AC021427_B (GPCR2a1), AC021427_B1 (GPCR2a2), AC021427_B_da1 (GPCR2a3), and AC021427_B_da2 (GPCR2a4). A first nucleic acid, AC021427_B (GPCR2a1), is 984 nucleotides long (SEQ ID NO:3). An open reading frame was identified beginning with an ATG initiation codon at nucleotides 24-26 and ending with a TGA codon at nucleotides 957-959. Putative untranslated regions upstream from the initiation codon and downstream from the termination codon are underlined in Table 2A, and the start and stop codons are in bold letters. The encoded protein having 311 amino acid residues is presented using the one-letter code in Table 2B (SEQ ID NO:4).

Table 2A. GPCR2a1 Nucleotide Sequence (SEQ ID NO:3).

AGTTGGCACCATTCCCAATATAG**ATG**GGGACTGGAAATGACACCACTGTGGTAGAGTTTACTCTTTT
GGGGTTATCTGAGGATACTACAGTTTGTGCTATTTTATTTCTTGTGTTTCTAGGAATTTATGTTGTC
ACCTTAATGGGTAATATCAGCATAATTGTATTGATCAGAAGAAGTCATCATCTTCATACACCCATGT
ACATTTTCCTCTGCCATTTGGCCTTTGTAGACATTGGGTACTCCTCATCAGTCACACCTGTCATGCT
CATGAGCTTCCTAAGGAAAGAAACCTCTCTCCCTGTTGCTGGTTGTGTGGCCCAGCTCTGTTCTGTA
GTGACGTTTGGTACGGCCGAGTGCTTCCTGCTGGCTGCCATGGCCTATGATCGCTATGTGGCCATCT
GCTCACCCCTGCTCTACTCTACCTGCATGTCCCCTGGAGTCTGCATCATCTTAGTGGGCATGTCCTA
CCTGGGTGGATGTGTGAATGCTTGGACATTCATTGGCTGCTTATTAAGACTGTCCTTCTGTGGGCCA
AATAAAGTCAATCACTTTTTCTGTGACTATTCACCACTTTTGAAGCTTGCTTGTTCCCATGATTTTA
CTTTTGAAATAATTCCAGCTATCTCTTCTGGATCTATCATTGTGGCCACTGTGTGTGTCATAGCCAT
ATCCTACATCTATATCCTCATCACCATCCTGAAGATGCACTCCACCAAGGGCCGCCACAAGGCCTTC
TCCACCTGCACCTCCCACCTCACTGCAGTCACTCTGTTCTATGGGACCATTACCTTCATTTATGTGA
TGCCCAAGTCCAGCTACTCAACTGACCAGAACAAGGTGGTGTCTGTGTTCTACACCGTGGTGATTCC
CATGTTGAACCCCCTGATCTACAGCCTCAGGAACAAGGAGATTAAGGGGGCTCTGAAGAGAGAGCTT
AGAATAAAAATATTTTCT**TGA**TGAAACTAGTTAGTTTGAAGAATCT (SEQ ID NO:3).

The disclosed nucleic acid GPCR2a1 sequence has 771 of 966 bases (79%) identical (with 771/966 positives, 79%) to a 1267 bp *Mus musculus* odorant receptor S25 gene (GENBANK-ID: AF121799) ($E = 4.8e^{-130}$).

The GPCR2a polypeptide (SEQ ID NO:4) encoded by SEQ ID NO:3 is presented using the one-letter amino acid code in Table 2B. The Psort profile for GPCR2a predicts that this sequence has a signal peptide and is likely to be localized at the plasma membrane with a certainty of 0.6850. The most likely cleavage site for a GPCR2a peptide is between amino acids 41 and 42, *i.e.*, at the slash in the amino acid sequence LMG-NI, based on the SignalP result.

Table 2B. GPCR2a protein sequence (SEQ ID NO:4)

MGTGNDTTVVEFTLLGLSEDTTVCAILFLVFLGIYVVTLMG/NISIIVLIRRSHHLHTPMYIFLCHL
AFVDIGYSSSVTPVMLMSFLRKETSLPVAGCVAQLCSVVTFGTAECFLLAAMAYDRYVAICSPLLYS
TCMSPGVCIILVGMSYLGGCVNAWTFIGCLLRLSFCGPNKVNHFFCDYSPLLKLACSHDFTFEIIPA
ISSGSIIVATVCVIAISYIYILITILKMHSTKGRHKAFSTCTSHLTAVTLFYGTITFIYVMPKSSYS
TDQNKVVSVFYTVVIPMLNPLIYSLRNKEIKGALKRELRIKIFS

The full amino acid sequence of the disclosed GPCR2a polypeptide has 237 of 303 amino acid residues (79%) identical to, and 266 of 303 residues (87%) positive with, the 342 amino acid residue protein from *Mus musculus* (ptnr:SPTREMBL-ACC:Q9WU91, SEQ ID NO:25, see below). This represents 76% identity to the full length AC021427_B ($E = 1.3e^{-125}$). Other BLAST results, from the Patp database include those listed in Table 2C.

Table 2C. GPCR2a PatP results

Sequences producing High-scoring Segment Pairs:	Reading Frame	High Score	Smallest Sum Prob. P(N)
patp:Y90877 Human G protein-coupled receptor GTAR11-3 ..	+3	769	1.4e-75
patp:Y90876 Human G protein-coupled receptor GTAR11-2 ..	+3	756	3.4e-74
patp:Y90876 Human G protein-coupled receptor GTAR11-2 ..	+3	756	3.4e-74
patp:Y83394 Olfactory receptor protein OLF-9 - Homo sa..	+3	754	5.5e-74
patp:Y83387 Olfactory receptor protein OLF-2 - Homo sa..	+3	745	4.9e-73

For example, a BLAST against patp: Y90877, a 313 amino acid human G-coupled receptor protein, produced 150/305 (49%) identity, and 211/305 (69%) identity ($E = 1.4e^{-75}$); and a BLAST against patp: Y90876, a 313 amino acid human G-coupled receptor protein, produced 147/305 (49%) identity, and 208/305 (68%) identity ($E = 3.4e^{-74}$). (PCT publication WO 00/21999). These proteins show identity with conserved regions of GPCR2, for example amino acids 118-132 (MAYDRYVAICXPLLY, where X is N, K, or S (SEQ ID NO:26)) and 283-295 (PMLNPLIYSLRNK (SEQ ID NO: 27)), see Clustal W alignment, Table 2M, below.

The predicted GPCR2a sequence, above, was subjected to the exon linking process to confirm the sequence. PCR primers were designed by starting at the most upstream sequence available, for the forward primer, and at the most downstream sequence available for the reverse primer. In each case, the sequence was examined, walking inward from the respective termini toward the coding sequence, until a suitable sequence that is either unique or highly selective was encountered, or, in the case of the reverse primer, until the stop codon was reached. Such suitable sequences were then used as the forward and reverse primers in a PCR amplification based on a wide range of cDNA libraries. The resulting amplicon was gel purified, cloned and sequenced to high redundancy, as described in the Examples.

The cloned sequence is disclosed as an alternative embodiment of GPCR2a (SEQ ID NO:5), referred to herein as the GPCR2a2 and reported in Table 2D. This 952 nucleotide sequence (GPCR2a2) is alternatively referred to herein as AC021427_B1. This nucleic acid is shorter than GPCR2a1 by 17 nucleotides in the 5'UTR, and by 15 nucleotides at the 3'UTR. Additionally, there are three different nucleotides: T77 is C60 in GPCR2a2, A335 is G316 in GPCR2a2, and C776 is T759 is GPCR2a2. Each of these changes is silent, and CPCR2a2 encodes the same 311 amino acid protein (GPCR2a, SEQ ID NO:4).

Table 2D. GPCR2a2 Nucleotide Sequence (SEQ ID NO:5)

ATATAGATGGGGACTGGAAATGACACCACTGTGGTAGAGTTTACTCTTTTGGGGTTATCCGAGGATA
CTACAGTTTGTGCTATTTTATTTCTTGTGTTTCTAGGAATTTATGTTGTCACCTTAATGGGTAATAT
CAGCATAATTGTATTGATCAGAAGAAGTCATCATCTTCATACACCCATGTACATTTTCCTCTGCCAT
TTGGCCTTTGTAGACATTGGGTACTCCTCATCAGTCACACCTGTCATGCTCATGAGCTTCCTAAGGA
AAGAAACCTCTCTCCCTGTTGCTGGTTGTGTGGCCCAGCTCTGTTCTGTGGTGACGTTTGGTACGGC
CGAGTGCTTCCTGCTGGCTGCCATGGCCTATGATCGCTATGTGGCCATCTGCTCACCCCTGCTCTAC
TCTACCTGCATGTCCCCTGGAGTCTGCATCATCTTAGTGGGCATGTCCTACCTGGGTGGATGTGTGA
ATGCTTGGACATTCATTGGCTGCTTATTAAGACTGTCCTTCTGTGGGCCAAATAAAGTCAATCACTT
TTTCTGTGACTATTCACCACTTTTGAAGCTTGCTTGTTCCCATGATTTTACTTTTGAAATAATTCCA
GCTATCTCTTCTGGATCTATCATTGTGGCCACTGTGTGTGTCATAGCCATATCCTACATCTATATCC
TCATCACCATCCTGAAGATGCACTCCACCAAGGGCCGCCACAAGGCCTTCTCCACCTGCACCTCCCA
CCTCACTGCAGTCACTCTGTTTTATGGGACCATTACCTTCATTTATGTGATGCCCAAGTCCAGCTAC
TCAACTGACCAGAACAAGGTGGTGTCTGTGTTCTACACCGTGGTGATTCCCATGTTGAACCCCCTGA
TCTACAGCCTCAGGAACAAGGAGATTAAGGGGGCTCTGAAGAGAGAGCTTAGAATAAAAATATTTTC
TTGATGAAACTAGT (SEQ ID NO: 5)

The target sequence identified as AC021427_B (GPCR2a1) was again subjected to the exon linking process to confirm the sequence. PCR primers were designed by starting at the most upstream sequence available, for the forward primer, and at the most downstream sequence available for the reverse primer. In each case, the sequence was examined, walking inward from the respective termini toward the coding sequence, until a suitable sequence that is either unique or highly selective was encountered, or, in the case of the reverse primer, until the stop codon was reached. Such primers were designed based on *in silico* predictions for the full length cDNA, part (one or more exons) of the DNA or protein sequence of the target sequence, or by translated homology of the predicted exons to closely related human sequences from other species. These primers were then employed in PCR amplification based on the following pool of human cDNAs: adrenal gland, bone marrow, brain (amygdala, cerebellum, hippocampus, substantia nigra, thalamus, and whole), fetal brain, fetal kidney, fetal liver, fetal lung, heart, kidney, lymphoma - Raji, mammary gland, pancreas, pituitary gland, placenta, prostate, salivary gland, skeletal muscle, small intestine, spinal cord, spleen, stomach, testis, thyroid, trachea, uterus. Typically, the resulting amplicons were gel purified, cloned and sequenced to high redundancy as described in the examples. The resulting sequences from all clones were assembled with themselves, with other fragments in CuraGen Corporation's database and with public ESTs. Fragments and ESTs were included as components for an assembly when the extent of their identity with another component of the assembly was at least 95% over 50 bp. In addition, sequence traces were evaluated manually and edited for corrections if appropriate. These procedures provided a third nucleic acid encoding GPCR2a, which is referred to as GPCR2a3, or AC021427_B_da1. This nucleic acid

is 964 nucleotides long (SEQ ID NO:8, Table 2E) and is 16 nucleotides shorter in the 5' UTR, and 4 nucleotides shorter in the 3'UTR than GPCR2a1. Silent base substitutions are: A17 is T1 in GPCR2a3, A335 is G319 in GPCR2a3; C776 is T760 in GPCR2a3.

Table 2E. GPCR2a3 Nucleotide Sequence (SEQ ID NO:8)

```
TATATAGATGGGGACTGGAAATGACACCACTGTGGTAGAGTTTACTCTTTTGGGGTTATCCGAGGAT
ACTACAGTTTGTGCTATTTTATTTCTTGTGTTTCTAGGAATTTATGTTGTCACCTTAATGGGTAATA
TCAGCATAATTGTATTGATCAGAAGAAGTCATCATCTTCATACACCCATGTACATTTTCCTCTGCCA
TTTGGCCTTTGTAGACATTGGGTACTCCTCATCAGTCACACCTGTCATGCTCATGAGCTTCCTAAGG
AAAGAAACCTCTCTCCCTGTTGCTGGTTGTGTGGCCCAGCTCTGTTCTGTGGTGACGTTTGGTACGG
CCGAGTGCTTCCTGCTGGCTGCCATGGCCTATGATCGCTATGTGGCCATCTGCTCACCCCTGCTCTA
CTCTACCTGCATGTCCCCTGGAGTCTGCATCATCTTAGTGGGCATGTCCTACCTGGGTGGATGTGTG
AATGCTTGGACATTCATTGGCTGCTTATTAAGACTGTCCTTCTGTGGGCCAAATAAAGTCAATCACT
TTTTCTGTGACTATTCACCACTTTTGAAGCTTGCTTGTTCCCATGATTTTACTTTTGAAATAATTCC
AGCTATCTCTTCTGGATCTATCATTGTGGCCACTGTGTGTGTCATAGCCATATCCTACATCTATATC
CTCATCACCATCCTGAAGATGCACTCCACCAAGGGCCGCCACAAGGCCTTCTCCACCTGCACCTCCC
ACCTCACTGCAGTCACTCTGTTTTATGGGACCATTACCTTCATTTATGTGATGCCCAAGTCCAGCTA
CTCAACTGACCAGAACAAGGTGGTGTCTGTGTTCTACACCGTGGTGATTCCCATGTTGAACCCCCTG
ATCTACAGCCTCAGGAACAAGGAGATTAAGGGGGCTCTGAAGAGAGAGCTTAGAATAAAAATATTTT
CTTGATGAAACTAGTTAGTTTGAAGA
```

A fourth nucleic acid encoding GPCR2a resulted from the exon linking procedure. This nucleic acid is referred to herein as GPCR2a4 or AC 021427_B_da2 (SEQ ID NO:9, Table 2F). This nucleic acid is 1604 nucleotides long. The 5'UTR is 624 nucleotides longer than GPCR2a1, and the 3'UTR is 4 nucleotides shorter. A single, silent nucleotide substitution is T77 to C701 in GPCR2a4.

Table 2F. GPCR2a4 Nucleotide Sequence (SEQ ID NO:9)

```
ACTCACTATAGGGCTCGAGCGGCCGCCCGGGCAGGTTGGATATTTGGTGTACTTTTCCTCAATTGTGAAATCTCTGGGTG
GGGACCACAGCTCAGTGTTGAGTTACTGACCTCTTCTTGGTCCTGTGGATTAGCATGCAGCTAATCTGTTTGACCTCTGT
TTGGAATTCGGAATTCTTAATGACTACACATCTTTGATACAATAGATGATACCTCAAACATCCTTTTGAACAGCTGTTCT
TTTCCATGAGTCTTGGTCTATTCTGACATTTATGTCTCCCTTCAATCACGTCTTTGGCCTTAGAAGATTGAGTTACTGGA
TTCTTTATATATTCTAGTGGTCATCTCTGAAATGTGCTCAGAGAGCACCTAAATTAACCATCCAATACGAGTTGAGTGTG
TTAAGTTAAAAAAAAAAAAAAAGATTTTTCTGAGTATTCCTGACCTTACATCAGTGAACATTTATGTTTTAAAGTCTTACA
TAAGATACTGTGTGTGAAAGCATTTTCTTCCCAAATTTACATGAGTGCCTAAATTGTTATACTTTTTGGTTAAATAGATA
TTGGAAAAATAAGTGTGCAATTATAGCATTTAATCCCATTATAATATTCATTTGTTTTTCTTTCAGTTGGCACCATTCCC
AATATAGATGGGGACTGGAAATGACACCACTGTGGTAGAGTTTACTCTTTTGGGGTTATCCGAGGATACTACAGTTTGTG
CTATTTTATTTCTTGTGTTTCTAGGAATTTATGTTGTCACCTTAATGGGTAATATCAGCATAATTGTATTGATCAGAAGA
AGTCATCATCTTCATACACCCATGTACATTTTCCTCTGCCATTTGGCCTTTGTAGACATTGGGTACTCCTCATCAGTCAC
ACCTGTCATGCTCATGAGCTTCCTAAGGAAAGAAACCTCTCTCCCTGTTGCTGGTTGTGTGGCCCAGCTCTGTTCTGTAG
TGACGTTTGGTACGGCCGAGTGCTTCCTGCTGGCTGCCATGGCCTATGATCGCTATGTGGCCATCTGCTCACCCCTGCTC
TACTCTACCTGCATGTCCCCTGGAGTCTGCATCATCTTAGTGGGCATGTCCTACCTGGGTGGATGTGTGAATGCTTGGAC
ATTCATTGGCTGCTTATTAAGACTGTCCTTCTGTGGGCCAAATAAAGTCAATCACTTTTTCTGTGACTATTCACCACTTT
TGAAGCTTGCTTGTTCCCATGATTTTACTTTTGAAATAATTCCAGCTATCTCTTCTGGATCTATCATTGTGGCCACTGTG
TGTGTCATAGCCATATCCTACATCTATATCCTCATCACCATCCTGAAGATGCACTCCACCAAGGGCCGCCACAAGGCCTT
CTCCACCTGCACCTCCCACCTCACTGCAGTCACTCTGTTCTATGGGACCATTACCTTCATTTATGTGATGCCCAAGTCCA
GCTACTCAACTGACCAGAACAAGGTGGTGTCTGTGTTCTACACCGTGGTGATTCCCATGTTGAACCCCCTGATCTACAGC
CTCAGGAACAAGGAGATTAAGGGGGCTCTGAAGAGAGAGCTTAGAATAAAAATATTTTCTTGATGAAACTAGTTAGTTTG
AAGA
```

GPCR2b

As described above and elaborated on in the Examples, AC021427_B, was subjected to the exon linking process to confirm the sequence. The resulting amplicon was gel purified, cloned and sequenced to high redundancy to provide GPCR2b (AC021427_B2, SEQ ID NO:6, Table 2G). The nucleic acid is 984 nucleotides, and differs from GPCR2a1 at 6 positions: G71A, T77C, A335G, C434T, C763T, and C776T. The encoded protein is 311 amino acids and differs from GPCR2a at position 247, where alanine is replaced with a valine. GPCR2b is shown in Table 2H, and is given SEQ ID NO:7.

Table 2G. GPCR2b Nucleotide Sequence (SEQ ID NO:6)

AGTTGGCACCATTCCCAATATAGATGGGGACTGGAAATGACACCACTGTGGTAGAGTTTACTCTTTTGGGATTATCCGAG
GATACTACAGTTTGTGCTATTTTATTTCTTGTGTTTCTAGGAATTTATGTTGTCACCTTAATGGGTAATATCAGCATAAT
TGTATTGATCAGAAGAAGTCATCATCTTCATACACCCATGTACATTTTCCTCTGCCATTTGGCCTTTGTAGACATTGGGT
ACTCCTCATCAGTCACACCTGTCATGCTCATGAGCTTCCTAAGGAAAGAAACCTCTCTCCCTGTTGCTGGTTGTGTGGCC
CAGCTCTGTTCTGTGGTGACGTTTGGTACGGCCGAGTGCTTCCTGCTGGCTGCCATGGCCTATGATCGCTATGTGGCCAT
CTGCTCACCCCTGCTCTACTCTACCTGCATGTCTCCTGGAGTCTGCATCATCTTAGTGGGCATGTCCTACCTGGGTGGAT
GTGTGAATGCTTGGACATTCATTGGCTGCTTATTAAGACTGTCCTTCTGTGGGCCAAATAAAGTCAATCACTTTTTCTGT
GACTATTCACCACTTTTGAAGCTTGCTTGTTCCCATGATTTTACTTTTGAAATAATTCCAGCTATCTCTTCTGGATCTAT
CATTGTGGCCACTGTGTGTGTCATAGCCATATCCTACATCTATATCCTCATCACCATCCTGAAGATGCACTCCACCAAGG
GCCGCCACAAGGCCTTCTCCACCTGCACCTCCCACCTCACTGTAGTCACTCTGTTTTATGGGACCATTACCTTCATTTAT
GTGATGCCCAAGTCCAGCTACTCAACTGACCAGAACAAGGTGGTGTCTGTGTTCTACACCGTGGTGATTCCCATGTTGAA
CCCCCTGATCTACAGCCTCAGGAACAAGGAGATTAAGGGGGCTCTGAAGAGAGAGCTTAGAATAAAAATATTTTCTTGAT
GAAACTAGTTAGTTTGAAGAATCT

Table 2H. GPCR2b Amino Acid Sequence (SEQ ID NO:7)

MGTGNDTTVVEFTLLGLSEDTTVCAILFLVFLGIYVVTLMGNISIIVLIRRSHHLHTPMYIFLCHLAF
VDIGYSSSVTPVMLMSFLRKETSLPVAGCVAQLCSVVTFGTAECFLLAAMAYDRYVAICSPLLYSTCM
SPGVCIILVGMSYLGGCVNAWTFIGCLLRLSFCGPNKVNHFFCDYSPLLKLACSHDFTFEIIPAISSG
SIIVATVCVIAISYIYILITILKMHSTKGRHKAFSTCTSHLTVVTLFYGTITFIYVMPKSSYSTDQNK
VVSVFYTVVIPMLNPLIYSLRNKEIKGALKRELRIKIFS

BlastP results for GPCR2b include: ptnr:SPTREMBL-ACC:Q9WU91 ODORANT RECEPTOR S25 - Mus musculus (Mouse), 342 aa., Score = 1241 (436.9 bits), Expect = 3.2e-126, P = 3.2e-126 Identities = 236/303 (77%), Positives = 266/303 (87%); ptnr:SPTREMBL-ACC:Q90808 OLFACTORY RECEPTOR 4 - Gallus gallus (Chicken), 312 amino acids(fragment).Score = 806 (283.7 bits), Expect = 4.0e-80, P = 4.0e-80 Identities = 166/310 (53%), Positives = 210/310 (67%); ptnr:SPTREMBL-ACC:O77757 OLFACTORY RECEPTOR OR93OO - Pongo pygmaeus (Orangutan), 313 amino acids, Score = 796 (280.2 bits), Expect = 4.6e-79, P = 4.6e-79 Identities = 162/303 (53%), Positives = 201/303 (66%).

The most homologous human sequence identified is ptnr:SWISSNEW-ACC:Q13606 OLFACTORY RECEPTOR 5I1 (OLFACTORY RECEPTOR-LIKE PROTEIN OLF1) - Homo sapiens (Human), 314 aa, Score = 761 (267.9 bits), Expect = 2.3e-75, P = 2.3e-75 Identities = 147/307 (47%), Positives = 203/307 (66%).

A non redundant BLASTX gave the following results:

Table 2I. GPCR2b blasts

Identifier	Description	Frame	Score	E
ptnr:SPTREMBL-ACC:Q9WU91	ODORANT RECEPTOR S25 - Mus mu...	+3	1241	1.9e-125
ptnr:SPTREMBL-ACC:Q90808	OLFACTORY RECEPTOR 4 - Gallus..	. +3	806	2.4e-79
ptnr:SPTREMBL-ACC:O77757	OLFACTORY RECEPTOR OR93OO - P...	+3	796	2.7e-78
ptnr:SPTREMBL-ACC:Q90806	OLFACTORY RECEPTOR 2 - Gallus..	. +3	791	9.3e-78
ptnr:SPTREMBL-ACC:O77758	OLFACTORY RECEPTOR OR93GIB - ..	. +3	788	1.9e-77
ptnr:SPTREMBL-ACC:O77756	OLFACTORY RECEPTOR OR93CH - P...	+3	787	2.5e-77
ptnr:SPTREMBL-ACC:Q62943	TASTE BUD RECEPTOR PROTEIN TB...	+3	782	8.4e-77

GPCR2c

The exon linking process was used to determine an additional GPCR2 nucleic acid, referred to herein as GPCR2c or AC021427_B_da3. The disclosed nucleic acid is 1064 nucleotides long, and is shown in Table 2H as SEQ ID NO:10. GPCR2c has 624 additional nucleotides in the 5' UTR and 4 nucleotides fewer at the 3' UTR when compared to GPCR2a1. There are four nucleotide substitutions relative to GPCR2a1: A19 is T644 in GPCR2c; G70 is A695 in GPCR2c; T76 is C701 in GPCR2c; and G302 is A927 in GPCR2c. The GPCR2c protein has 311 amino acids and differs from CPCR2a at position 94, where valine has been changed to isoleucine. It is understood that the GPCR2 nucleic acids include nucleic acids that vary at one or more of these nucleotides. The amino acid sequence for GPCR2c is shown in Table 2I as SEQ ID NO:11.

Table 2J. GPCR2c Nucleotide Sequence (SEQ ID NO:10)

```
ACTCACTATAGGGCTCGAGCGGCCGCCCGGGCAGGTTGGATATTTGGTGTACTTTTCCTCAATTGTGAAATCTCTGGG
TGGGGACCACAGCTCAGTGTTGAGTTACTGACCTCTTCTTGGTCCTGTGGATTAGCATGCAGCTAATCTGTTTGACCT
CTGTTTGGAATTCGGAATTCTTAATGACTACACATCTTTGATACAATAGATGATACCTCAAACATCCTTTTGAACAGC
TGTTCTTTTCCATGAGTCTTGGTCTATTCTGACATTTATGTCTCCCTTCAATCACGTCTTTGGCCTTAGAAGATTGAG
TTACTGGATTCTTTATATATTCTAGTGGTCATCTCTGAAATGTGCTCAGAGAGCACCTAAATTAACCATCCAATACGA
GTTGAGTGTGTTAAGTTAAAAAAAAAAAAAAAGATTTTTCTGAGTATTCCTGACCTTACATCAGTGAACATTTATGTTT
TAAAGTCTTACATAAGATACTGTGTGTGAAAGCATTTTCTTCCCAAATTTACATGAGTGCCTAAATTGTTATACTTTT
TGGTTAAATAGATATTGGAAAAATAAGTGTGCAATTATAGCATTTAATCCCATTATAATATTCATTTGTTTTTCTTTC
AGTTGGCACCATTCCCAATTTAGATGGGGACTGGAAATGACACCACTGTGGTAGAGTTTACTCTTTTGGGATTATCCG
AGGATACTACAGTTTGTGCTATTTTATTTCTTGTGTTTCTAGGAATTTATGTTGTCACCTTAATGGGTAATATCAGCA
TAATTGTATTGATCAGAAGAAGTCATCATCTTCATACACCCATGTACATTTTCCTCTGCCATTTGGCCTTTGTAGACA
TTGGGTACTCCTCATCAGTCACACCTGTCATGCTCATGAGCTTCCTAAGGAAAGAAACCTCTCTCCCTATTGCTGGTT
GTGTGGCCCAGCTCTGTTCTGTAGTGACGTTTGGTACGGCCGAGTGCTTCCTGCTGGCTGCCATGGCCTATGATCGCT
ATGTGGCCATCTGCTCACCCCTGCTCTACTCTACCTGCATGTCCCCTGGAGTCTGCATCATCTTAGTGGGCATGTCCT
ACCTGGGTGGATGTGTGAATGCTTGGACATTCATTGGCTGCTTATTAAGACTGTCCTTCTGTGGGCCAAATAAAGTCA
ATCACTTTTTCTGTGACTATTCACCACTTTTGAAGCTTGCTTGTTCCCATGATTTTACTTTTGAAATAATTCCAGCTA
TCTCTTCTGGATCTATCATTGTGGCCACTGTGTGTGTCATAGCCATATCCTACATCTATATCCTCATCACCATCCTGA
AGATGCACTCCACCAAGGGCCGCCACAAGGCCTTCTCCACCTGCACCTCCCACCTCACTGCAGTCACTCTGTTCTATG
GGACCATTACCTTCATTTATGTGATGCCCAAGTCCAGCTACTCAACTGACCAGAACAAGGTGGTGTCTGTGTTCTACA
CCGTGGTGATTCCCATGTTGAACCCCCTGATCTACAGCCTCAGGAACAAGGAGATTAAGGGGGCTCTGAAGAGAGAGC
TTAGAATAAAAATATTTTCTTGATGAAACTAGTTAGTTTGAAGA
```

Table 2K. GPCR2c Amino Acid Sequence (SEQ ID NO:11)

```
MGTGNDTTVVEFTLLGLSEDTTVCAILFLVFLGIYVVTLMGNISIIVLIRRSHHLHTPMYIFLCHLAFVDIGYSSSVT
PVMLMSFLRKETSLPIAGCVAQLCSVVTFGTAECFLLAAMAYDRYVAICSPLLYSTCMSPGVCIILVGMSYLGGCVNA
WTFIGCLLRLSFCGPNKVNHFFCDYSPLLKLACSHDFTFEIIPAISSGSIIVATVCVIAISYIYILITILKMHSTKGR
HKAFSTCTSHLTAVTLFYGTITFIYVMPKSSYSTDQNKVVSVFYTVVIPMLNPLIYSLRNKEIKGALKRELRIKIFS
```

The most homologous sequences identified (BLASTP Non-Redundant Composite database) include: ptnr:SPTREMBL-ACC:Q9WU91 ODORANT RECEPTOR S25 - *Mus musculus* (Mouse), 342 aa.; Score = 1238 (435.8 bits), Expect = 6.4e-126, P = 6.4e-126, Identities = 236/303 (77%), Positives = 265/303 (87%); ptnr:SPTREMBL-ACC:Q90808 OLFACTORY RECEPTOR 4 - *Gallus gallus* (Chicken), 312 amino acids(fragment), Score = 801 (282.0 bits), Expect = 1.3e-79, P = 1.3e-79, Identities = 165/310 (53%), Positives = 209/310 (67%).

The most homologous human sequence identified is ptnr:SWISSPROT-ACC:Q13606 OLFACTORY RECEPTOR-LIKE PROTEIN OLF1 - *Homo sapiens* (Human), 314 aa, Score = 758 (266.8 bits), Expect = 4.7e-75, P = 4.7e-75, Identities = 147/307 (47%), Positives = 202/307 (65%).

Table 2L. GPCR2c blasts

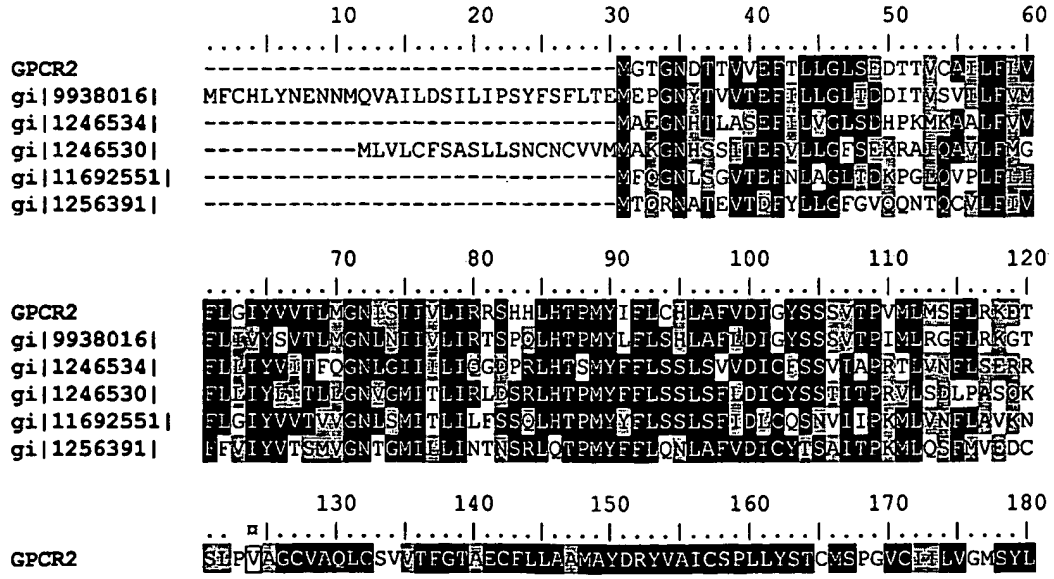
Identifier	Description	Frame	score	E
ptnr:SPTREMBL-ACC:Q9WU91	ODORANT RECEPTOR S25 - Mus mus...	+1	1242	1.5e-125
ptnr:SPTREMBL-ACC:Q90808	OLFACTORY RECEPTOR 4 - Gallus..	. +1	805	3.0e-79
ptnr:SPTREMBL-ACC:O77757	OLFACTORY RECEPTOR OR93OO - P...	+1	795	3.4e-78
ptnr:SPTREMBL-ACC:Q90806	OLFACTORY RECEPTOR 2 - Gallus..	. +1	787	9.0e-78
ptnr:SPTREMBL-ACC:O77758	OLFACTORY RECEPTOR OR93GIB - ..	. +1	786	2.4e-77
ptnr:SPTREMBL-ACC:O77756	OLFACTORY RECEPTOR OR93CH - P...	+1	782	3.1e-77
ptnr:SPTREMBL-ACC:Q62943	TASTE BUD RECEPTOR PROTEIN TB...	+1	774	8.1e-77

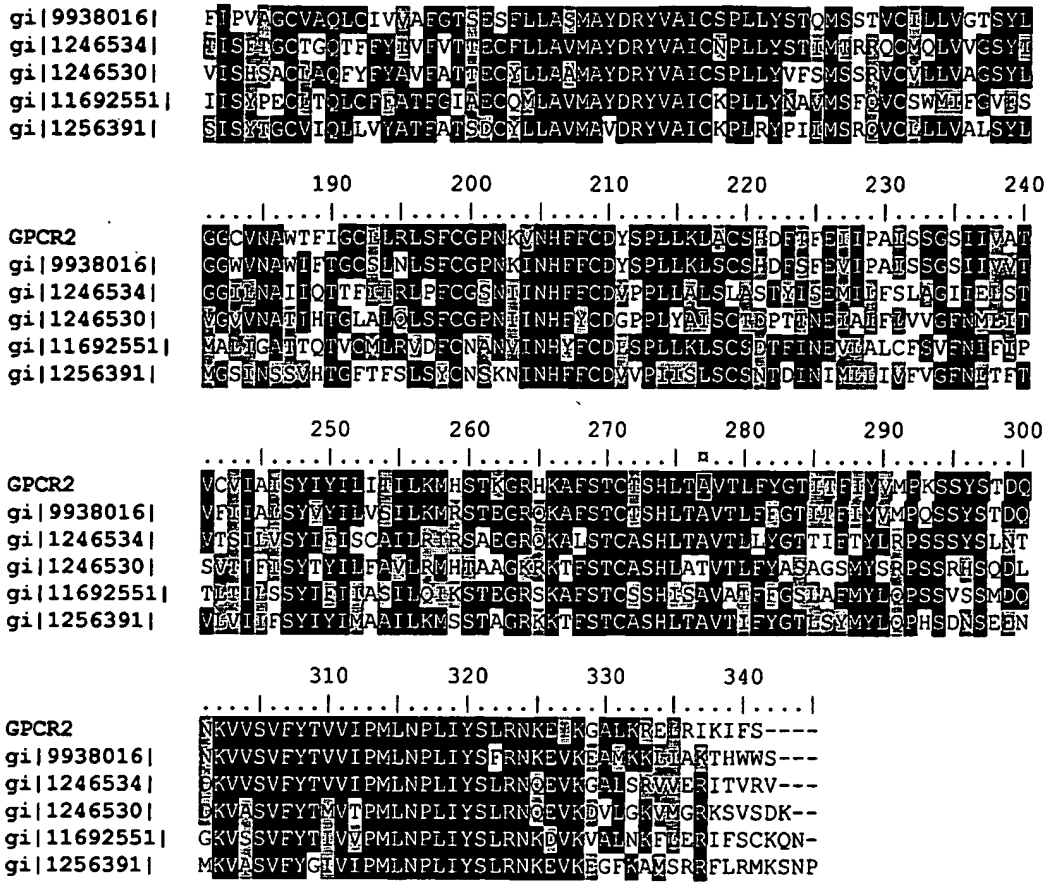
Unless specifically addressed as GPCR2a GPCR2b or GPCR2c, any reference to GPCR2 is assumed to encompass all variants. Residue differences between any GPCRX variant sequences herein are written to show the residue in the "a" variant and the residue position with respect to the "a" variant. GPCR2 residues in all following sequence alignments that differ between the individual GPCR2 variants are highlighted with a box and marked with the (o) symbol above the variant residue in all alignments herein.

A multiple sequence alignment is given in Table 2M, with the GPCR2 protein of the invention being shown on line 1, in a ClustalW analysis comparing GPCR2 with related protein sequences.

Table 2M Information for the ClustalW proteins:

1) GPCR2 (SEQ ID NO:4)
2) gi|9938016|ref|NP_064687.1| odorant receptor S25 gene [Mus musculus] (SEQ ID NO:25)
3) gi|1246534|emb|CAA64370.1| olfactory receptor 4 [Gallus gallus] (SEQ ID NO:21)
4) gi|1246530|emb|CAA64368.1| olfactory receptor 2 [Gallus gallus] (SEQ ID NO:20)
5) gi|11692551|gb|AAG39872.1|AF282287_1 odorant receptor K31 [Mus musculus] (SEQ ID NO:28)
6) gi|1256391|gb|AAC52910.1| taste bud receptor protein TB 567 (SEQ ID NO:29)



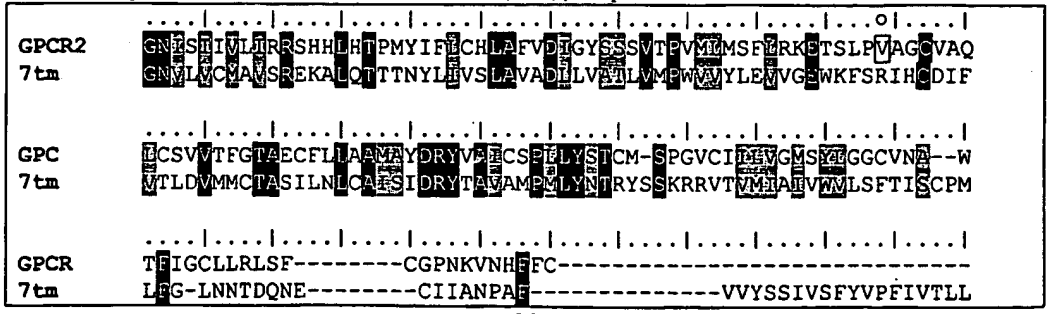


DOMAIN results for GPCR2 were collected from the Conserved Domain Database (CDD) with Reverse Position Specific BLAST. This BLAST samples domains found in the Smart and Pfam collections. The results are listed in Table 2J with the statistics and domain description. The 7tm_1, a seven transmembrane receptor (rhodopsin family), was shown to have homology to GPCR2. An alignment of the disclosed GPCR2a with residues 41-157 of GPCR2 are shown in Table 2N.

Table 2N. DOMAIN results for GPCR2

gnl|Pfam|pfam00001, 7tm_1, 7 transmembrane receptor (rhodopsin family) (SEQ ID NO:24)

Length = 377; Score = 92.0 bits (227), Expect = 4e-20



The nucleic acids and proteins of GPCR2 are useful in potential therapeutic applications implicated in various GPCR-related pathological disorders and/or OR-related pathological disorders, described further below. For example, a cDNA encoding the olfactory receptor-like protein may be useful in gene therapy, and the olfactory receptor-like protein may be useful when administered to a subject in need thereof. By way of nonlimiting example, the compositions of the present invention will have efficacy for treatment of patients suffering from neoplasm, adenocarcinoma, lymphoma, prostate cancer, uterus cancer, immune response, AIDS, asthma, Crohn's disease, multiple sclerosis, and Albright Hereditary Ostoeodystrophy. Other GPCR-1 diseases and disorders are contemplated.

The novel nucleic acid encoding GPCR2, or fragments thereof, may further be useful in diagnostic applications, wherein the presence or amount of the nucleic acid or the protein are to be assessed. These materials are further useful in the generation of antibodies that bind immuno-specifically to the novel GPCR1 substances for use in therapeutic or diagnostic methods. These antibodies may be generated according to methods known in the art, using prediction from hydrophobicity charts, as described in the "Anti-GPCRX Antibodies" section below. In one embodiment, a contemplated GPCR2 epitope is from about amino acids 160 to 180. In another embodiment, a GPCR2 epitope is from about amino acids 230- to 250. In additional embodiments, GPCR2 epitopes are from amino acids 260 to 280 and from amino acids 290 to 310. These novel proteins can also be used to develop assay system for functional analysis.

GPCR3

An additional GPCR-like protein of the invention, referred to herein as GPCR3, is an Olfactory Receptor ("OR")-like protein. The novel nucleic acid of 1028 nucleotides (AC021427_C, SEQ ID NO:12) encoding a novel G-protein coupled receptor-like protein is shown in Table 3A. An open reading frame (ORF) was identified beginning with an ATG initiation codon at nucleotides 23-25 and ending with a TAA codon at nucleotides 1007-1009. Putative untranslated regions upstream from the initiation codon and downstream from the termination codon are underlined in Table 3A, and the start and stop codons are in bold letters.

Table 3A. GPCR3 Nucle tide Sequence (SEQ ID NO:12)

AGTGGCCGTGCCCACCAGGAGGATGGGCAATCACACTGCAGTGAGCCTATTCCTTCTGTGGGGATTTT
CCAGTTTTTCAGACCTGCAGAGTCTACTTTGTGGTGATTCTCTTCTACATGTGACCATCCTAGCTGCA
AACGTGTCCATAATGGGGGCCATCAAGCTCAGCCACAACCTTCACACTCCTATGTACTTTTTCCTCTG
TGGCCTGTCCTTTTCAGAAACTTGTACCACTGTGGTAGTAATCCCTCGCATGTTGGTGGACTTTCTAT
CAGAGAGCAAGACCATTTCTCTTCCTGAGTGTGCCACACAGATGTTTTTCTTTCTGGGCTTTGCATCC
AACAACTGTTTCATCATGGCCGCTATGTCCTACGACCGCTACACGGCCATCCACAACCCACTGCAGTA
CCACACCCTTATGACAAGAAAGATCTGCTTGCAGATGATGATGGCTTCTTGGATGGTTGGGTTCCTGT
TTTCTCTGTGCATCATCGTCACTGTATTCAACTTGTCTCTTTGCGACTTGAACACTATCCAGCACTAT
TTCTGTGATATCTCACCAGTGGTCTCCCTTGCTTGTAATTACACTTTCTATCATGAAATGGCTATTTT
TGTGCTCTCTGCCTTTGTGTTGGTGGGCAGCTGTATTTTAATTATGATTTCCTATGTCTTCATTGTGT
TCATAGTCATAAAGATGCCCTCTGCAAAGGGGAGGTCTAAGGCCTTCTCAACTTGCTCCTCCCACCTC
ACTGTTGTGTCCATACACTATGGATTTGCTTGCTTTGTCTATTTGAGGCCCAAGAACAGCAACTCCTT
CGATGAAGACATGCTGACGGCCATGATATATACAATACTGATGCCTCTGCTTAACCCCATCGTGTACA
GTCTGAGAAACAAAGAAATGCAGATAGCCCTAAGAAAAACACTAGGCAGTGTATTTGGGGTTTTCCCT
CAGAAGACAAAAAAAGAGCCTGAACATTTAAAAAAATTACACAGCATTGATAAATAAAGGTGAGAAAA
GTGGAGTA

The disclosed AC021427_C nucleic acid sequence has 539 of 855 bases (63%) identical to an olfactory receptor mRNA (GENBANK-ID: X64995) from *Homo sapiens* (E= 1.4 e^{-41}).

The disclosed GPCR3 polypeptide (SEQ ID NO:13) encoded by SEQ ID NO:12 is 328 amino acid residues and is presented using the one-letter code in Table 3B. The GPCR3 protein was analyzed for signal peptide prediction and cellular localization. SignalP results predict that GPCR3 is cleaved between position 21 and 22 of SEQ ID NO:13, *i.e.*, at the slash in the amino acid sequence SDL-QS. Psort and Hydropathy profiles also predict that GPCR3 contains a signal peptide and is likely to be localized at the plasma membrane (certainty of 0.6760).

Table 3B. Encoded GPCR3 protein sequence (SEQ ID NO:13).

MGNHTAVSLFLLWGFSSFSDL/QSLLCGDSLLHVTILAANVSIMGAIKLSHNLHTPMYFFLCGLSFSE
TCTTVVVIPRMLVDFLSESKTISLPECATQMFFFLGFASNNCFIMAAMSYDRYTAIHNPLQYHTLMTR
KICLQMMMASWMVGFLFSLCIIVTVFNLSLCDLNTIQHYFCDISPVVSLACNYTFYHEMAIFVLSAFV
LVGSCILIMISYVFIVFIVIKMPSAKGRSKAFSTCSSHLTVVSIHYGFACFVYLRPKNSNSFDEDMLT
AMIYTILMPLLNPIVYSLRNKEMQIALRKTLGSVFGVFPQKTKKEPEHLKKLHSIDK

The full amino acid sequence of the protein of the invention was found to have 136 of 304 amino acid residues (44%) identical to, and 203 of 304 residues (66%) positive with, the 320 amino acid residue olfactory receptor-like protein HGMP07J (320 aa) from *Homo sapiens* (ptnr:SPTREMBL-ACC:P30954) (E = 3.9 e^{-79}). As shown in Table 3C, Patp blast analysis shows that GPCR3 has significant homology with a number of olfactory receptors.

Table 3C. PatP results

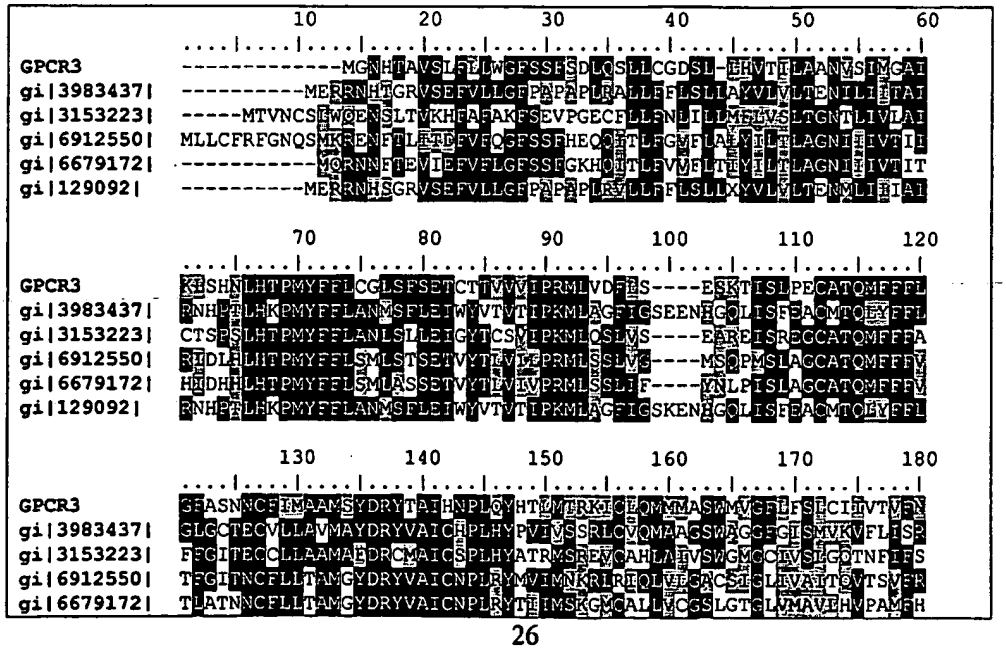
Sequences producing High-scoring Segment Pairs:	Reading Frame	High Score	Smallest Sum Prob. P(N)	N
patp:W21662 Rat spermatid chemoreceptor D-2 - Rattus s...	+2	649	7.3e-63	1
patp:R27872 Odorant receptor clone I7 - Rattus rattus,...	+2	648	9.4e-63	1
patp:Y83387 Olfactory receptor protein OLF-2 - Homo sa...	+2	621	6.8e-60	1
patp:W21664 Rat spermatid chemoreceptor D-8 - Rattus s...	+2	618	1.4e-59	1
patp:Y83389 Olfactory receptor protein OLF-4 - Homo sa...	+2	617	1.8e-59	1
patp:Y90877 Human G protein-coupled receptor GTAR11-3 ...	+2	615	2.9e-59	1

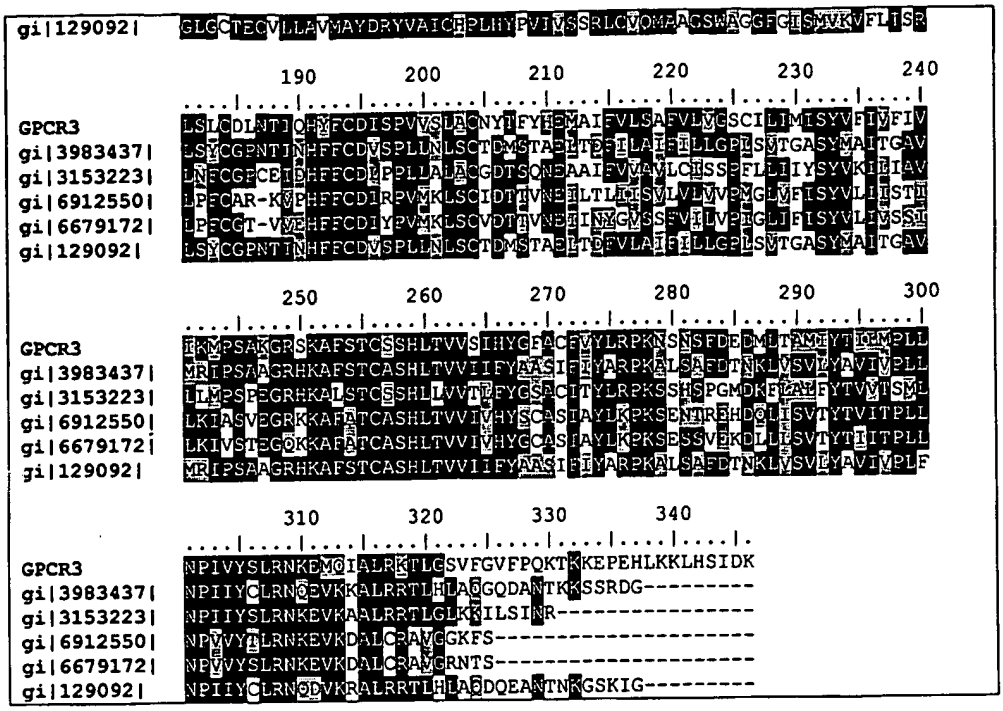
For example, a Blast of GPCR against patp:W21662, a 321 amino acid Rat spermatid chemoreceptor, shows that 137 of 295 amino acid residues (46%) are identical to, and 189 of 295 residues (64%) are positive with, this protein ($E = 7.3\ e^{-63}$). See, PCT publication WO 97/17444. A similar result was obtained from a blast of GPCR3 against Patp: Y83387 (PCT publication WO 00/21985). 124 of 306 amino acid residues (40%) are identical to, and 193 of 306 residues (63%) are positive with, the 315 amino acid residue olfactory receptor protein OLF2 from *Homo sapiens* ($E = 6.8\ e^{-60}$).

A multiple sequence alignment is given in Table 3D, with GPCR3 being shown on line 1, in a ClustalW analysis comparing GPCR3 with related protein sequences.

Table 3D. Information for the ClustalW proteins:

1) GPCR3 (SEQ ID NO: 13)
2) gi|3983437|gb|AAD13307.1| olfactory receptor I7 [Mus musculus] (SEQ ID NO: 30)
3) gi|3153223|gb|AAC17222.1| olfactory receptor-like protein [Rattus norvegicus] (SEQ ID NO: 31)
4) gi|6912550|ref|NP_036483.1| olfactory receptor, family 10, subfamily J, member 1 [Homo sapiens] (SEQ ID NO: 32)
5) gi|6679172|ref|NP_032789.1| olfactory receptor 16 [Mus musculus] (SEQ ID NO: 33)
6) gi|129092|sp|P23270|OLF7_RAT OLFACTORY RECEPTOR-LIKE PROTEIN I7 (SEQ ID NO: 34)





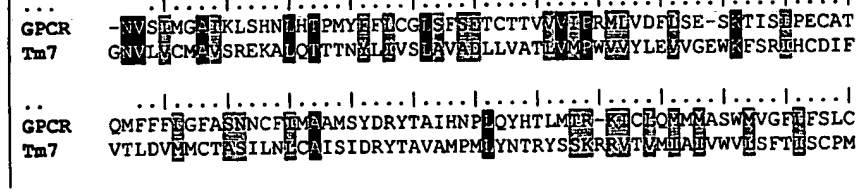
DOMAIN results for GPCR3 were collected from the Conserved Domain Database (CDD) with Reverse Position Specific BLAST. This BLAST samples domains found in the Smart and Pfam collections. The 7tm_1, a seven transmembrane receptor (rhodopsin family), was shown to have two segments with significant homology to GPCR3. The results are listed in Table 3E with the statistics and domain description. The region from amino acid residue 39 through 234 (numbered with respect to SEQ ID NO:13) aligns with amino acids 2 through 180 of the "seven transmembrane receptor (rhodopsin family) fragment" domain(SEQ ID NO:24), and GPCR3 amino acids 224-287 aligned with residues 313-377 of the 7tm_1 entry (SEQ ID NO:24) of the Pfam database. This indicates that the GPCR3 sequence has properties similar to those of other proteins known to contain this domain as well as to the 7tm_1 domain itself.

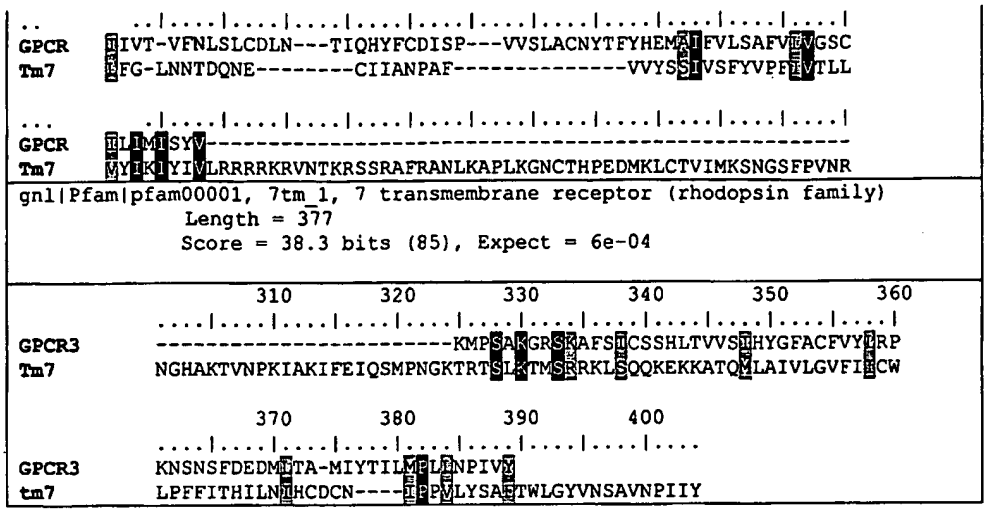
Table 3E. DOMAIN results for GPCR3.

gnl|Pfam|pfam00001, 7tm_1, 7 transmembrane receptor (rhodopsin family) (SEQ ID NO:23)

Length = 377

Score = 92.7 bits (220), Expect = 3e-20





The nucleic acids and proteins of the invention are useful in potential therapeutic applications implicated in various in various GPCR-related pathological disorders and/or OR-related pathological disorders, described further below. For example, a cDNA encoding the olfactory receptor -like protein may be useful in gene therapy, and the olfactory receptor -like protein may be useful when administered to a subject in need thereof. By way of nonlimiting example, the compositions of the present invention will have efficacy for treatment of patients suffering from neoplasm, adenocarcinoma, lymphoma, prostate cancer, uterus cancer, immune response, AIDS, asthma, Crohn's disease, multiple sclerosis, and Albright Hereditary Ostoeodystrophy. Other GPCR-related diseases and disorders are contemplated.

The novel nucleic acid encoding the GPCR-like protein of the invention, or fragments thereof, may further be useful in diagnostic applications, wherein the presence or amount of the nucleic acid or the protein are to be assessed. These materials are further useful in the generation of antibodies that bind immunospecifically to the novel substances of the invention for use in therapeutic or diagnostic methods. These antibodies may be generated according to methods known in the art, using prediction from hydrophobicity charts, as described in the "Anti-GPCRX Antibodies" section below. In one embodiment, a contemplated GPCR3 epitope is from about amino acids105 to 140. In another embodiment, a GPCR3 epitope is from about amino acids 250 to 275. In additional embodiments, GPCR3 epitopes are from amino acids 290 to 305 and from amino acids 310 to 330. These novel proteins can be used in assay systems for functional analysis of various human disorders, which will help in understanding of pathology of the disease and development of new drug targets for various disorders

GPCR4

The disclosed novel GPCR4 nucleic acid of 1070 nucleotides (also referred to as AC021427_D) is shown in Table 4A. An ORF begins with an ATG initiation codon at nucleotides 27-29 and ends with a TAG codon at nucleotides 1041-1043. A putative untranslated region upstream from the initiation codon and downstream from the termination codon is underlined in Table 4A, and the start and stop codons are in bold letters.

Table 4A. GPCR4 Nucleotide Sequence (SEQ ID NO:14)

GAAGCCATCAAATTTATAACATTTTA**ATG**TGCTGTTCTCATTTGGGTTCATTTAGTCAGCAGCTACT
TCGTCTCATGAATTCCCTGAAGGACGGGAATCACACCGCTCTGACGGGGTTCATCCTATTGGGCTTA
ACAGATGATCCAATCCTTCGAGTCATCCTCTTCATGATCATCCTATCTGGTAATCTCAGCATAATTA
TTCTTATCAGAATTTCTTCTCAGCTCCATCATCCTATGTATTTCTTTCTGAGCCACTTGGCTTTTGC
TGACATGGCCTATTCATCTTCTGTCACACCCAACATGCTTGTAAACTTCCTGGTGGAGAGAAATACA
GTCTCCTACCTTGGATGTGCCATCCAGCTTGGTTCAGCGGCTTTCTTTGCAACAGTCGAATGCGTCC
TTCTGGCTGCCATGGCCTATGACCGCTTTGTGGCAATTTGCAGTCCACTGCTTTATTCAACCAAAAT
GTCCACACAAGTCAGTGTCCAGCTACTCTTAGTAGTTTACATAGCTGGTTTTCTCATTGCTGTCTCC
TATACTACTTCCTTCTATTTTTTACTCTTCTGTGGACCAAATCAAGTCAATCATTTTTTCTGTGATT
TCGCTCCCTTACTTGAACTCTCCTGTTCTGATATCAGTGTCTCCACAGTTGTTCTCTCATTTTCTTC
TGGATCCATCATTGTGGTCACTGTGTGTGTCATAGCCGTCTGCTACATCTATATCCTCATCACCATC
CTGAAGATGCGCTCCACTGAGGGGCACCACAAGGCCTTCTCCACCTGCACTTCCCACCTCACTGTGG
TTACCCTGTTCTATGGGACCATTACCTTCATTTATGTGATGCCCAATTTTAGCTACTCAACTGACCA
GAACAAGGTGGTGTCTGTGTTGTACACAGTGGTGATTCCCATGTTGAACCCCCTGATCTACAGCCTC
AGGAACAAGGAGATTAAGGGGGCTCTGAAGAGAGAGCTTGTTAGAAAAATACTTTCTCATGATGCTT
GTTATTTTAGTAGAACTTCAAATAATGATATTACA**TAG**AACCCTATCTCTTCTCTTGAGAATACT

The GPCR4 protein encoded by SEQ ID NO:14 has 338 amino acid residues and is presented using the one-letter code in Table 4B. The Psort profile for GPCR4 predicts that this sequence has a signal peptide and is likely to be localized at the plasma membrane with a certainty of 0.6000. The most likely cleavage site for a peptide is between amino acids 65 and 66, *i.e.*, at the slash in the amino acid sequence ISS-QL, based on the SignalP result.

Table 4B. Encoded GPCR4 protein sequence (SEQ ID NO:15).

MCCSHLGSFSQQLLRLMNSLKDGNHTALTGFILLGLTDDPILRVILFMIILSGNLSIIILIRISS/
QLHHPMYFFLSHLAFADMAYSSSVTPNMLVNFLVERNTVSYLGCAIQLGSAAFFATVECVLLAAMA
YDRFVAICSPLLYSTKMSTQVSVQLLLVVYIAGFLIAVSYTTSFYFLLFCGPNQVNHFFCDFAPLL
ELSCSDISVSTVVLSFSSGSIIVVTVCVIAVCYIYILITILKMRSTEGHHKAFSTCTSHLTVVTLF
YGTITFIYVMPNFSYSTDQNKVVSVLYTVVIPMLNPLIYSLRNKEIKGALKRELVRKILSHDACYF
SRTSNNDIT

The disclosed nucleic acid sequence for GPCR4 has 635 of 894 bases (71%) identical to and 635 of 894 bases (71%) positive with *Mus musculus* odorant receptor S25 gene (1267 bp) (GENBANK-ID: AF121977) (E= $5.1e^{-78}$).

The full GPCR4 amino acid sequence has 193 of 310 amino acid residues (62 %) identical to, and 233 of 310 residues (73%) positive with, the 342 amino acid residue odorant receptor from *Mus musculus* (ptnr: SPTREMBL-ACC: Q9WU91) (E= $1.5e^{-92}$, see Clustal W analysis, below). BLAST analysis of the Patp database produced the significant results listed in Table 4C.

Table 4C Patp blast

Sequences producing High-scoring Segment Pairs:	Reading Frame	High Score	Smallest Sum Prob P(N)	N
patp:R27880 Pheromone receptor clone J4 - Rattus rattus..	+3	835	1.4e-82	1
patp:Y83389 Olfactory receptor protein OLF-4 - H.sapiens.	+3	753	7.0e-74	1
patp:Y83387 Olfactory receptor protein OLF-2 - H.sapiens.	+3	744	6.3e-73	1
patp:Y90877 Human G protein-coupled receptor GTAR11-3 ...	+3	720	2.2e-70	1

For example, as shown in Table 4D, the full GPCR4 amino acid sequence has 160 of 215 amino acid residues (74 %) identical to, and 183 of 215 residues (85%) positive with, the 215 amino acid residue pheromone receptor from *Rattus rattus* (ppat: R27880, PCT publication WO 92/17585) (E= $1.4e^{-82}$). This protein also has 152/302 amino acids identical to and 209/302 amino acids positive with (69%) Human olfactory receptor protein OLF-4 (patp Y83389, PCT publication WO 00/21985) (E= $7.0\ e^{-74}$).

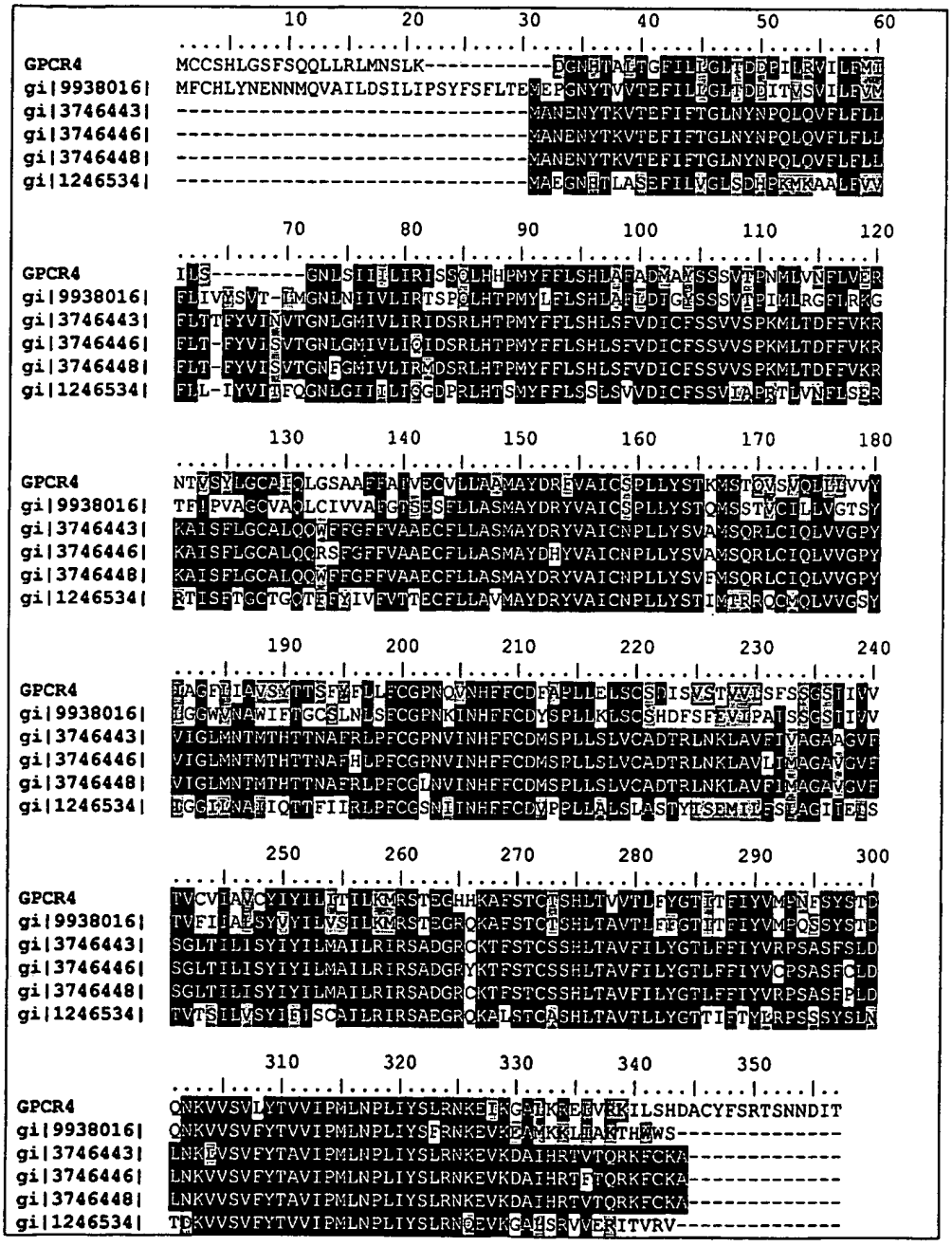
Table 4D. BLAST against Patp: R27880 (SEQ ID NO:35)

```
                10        20        30        40        50        60
                ....|....|....|....|....|....|....|....|....|....|....|
GPCR4           MCCSHLGSFSQQLLRLMNSLKDGNHTALTGFILLGLTDDPILRVILFMIILSGNLSIIIL 60
R27880          ------------------------------------------------------------

                        70        80        90       100       110       120
                ....|....|....|....|....|....|....|....|....|....|....|....|
GPCR4           IRISSQLHHPMYFFLSHLAFADMAYSSSVTPNMLVNFLVERNTVSYLGCAIQLGSAAFFA 120
R27880          ----------------------IGYSSSVTPNMLVNFLIKQNTISYLGCSIQFGSAAFFG 38

                       130       140       150       160       170       180
                ....|....|....|....|....|....|....|....|....|....|....|....|
GPCR4           TVECVLLAAMAYDRFVAICSPLLYSTKMSTQVSVQLLLVVYIAGFLIAVSYTTSFYFLF 180
R27880          ALECFLLAAMAYDRFVAICNPLLYSTKMSTQVCVQLVVGSYIGGFLNSSETLSFESLSF 98

                       190       200       210       220       230       240
                ....|....|....|....|....|....|....|....|....|....|....|....|
GOCR4           CGPNQVNHFFCDFAPLLELSCSDISVSTVVLSFSSGSFIVVTVCVIAVCMIYILITILKM 240
R27880          CGPNRINHFYCDFAPLVELSCSDVSVPDAVTSFSAASVTMLTVFIIAISYTYILITILKM 158

                       250       260       270       280       290       300
                ....|....|....|....|....|....|....|....|....|....|....|....|
GPCR4           RSTEGHHKAFSTCTSHLTVVTLFYGTITFIYVMPNFSYSTDQNKVVSVLYTVVIPMLNPL 300
R27880          RSTEGRQKAFSTCTSHLTAVTLCYGTITFIYVMPKSSYSTDQNKVVSVFYMVVIPML--- 215

                       310       320       330
                ....|....|....|....|....|...
GPCR            IYSLRNKEIKGALKRELVRKILSHDACYFSRTSNNDIT 338
R27880          -------------------------------------
```

A multiple sequence alignment is given in Table 4E, with the disclosed GPCR4 being shown on line 1, in a ClustalW analysis comparing GPCR4 with related protein sequences.

Table 4E. Information for the ClustalW proteins:

1) GPCR4 (SEQID NO:15)
2) gi|9938016|ref|NP_064687.1| odorant receptor S25 gene [Mus musculus] (SEQID NO:25)
3) gi|3746443|gb|AAC63969.1| olfactory receptor OR93Ch [Pan troglodytes] (SEQID NO:23)
4) gi|3746446|gb|AAC63970.1| olfactory receptor OR93Oo [Pongo pygmaeus] (SEQID NO:36)
5) gi|3746448|gb|AAC63971.1| olfactory receptor OR93Gib [Hylobates lar] (SEQID NO:22)
6) gi|1246534|emb|CAA64370.1| olfactory receptor 4 [Gallus gallus] (SEQID NO:21)



DOMAIN results for GPCR4 were collected from the Conserved Domain Database (CDD) with Reverse Position Specific BLAST. This BLAST samples domains found in the Smart and Pfam collections. The results are listed in Table 4D with the statistics and domain description.

Residues 16-97 and residues 313-377 of 7tm_1 are (SEQ ID NO:24) aligned with GPCR4 (68-149 and 236-302) in Table 4F.

Table 4F. DOMAIN results for GPCR4.

```
gnl|Pfam|pfam00001, 7tm_1, 7 transmembrane receptor (rhodopsin family)
(SEQ ID NO: 23)  Length = 377
  Statistics for GPCR4:   Score = 71.2 bits (173), Expect = 8e-14

...          .|....|....|....|....|....|....|....|....|....|....|....|
GPCR4     --------------HHPMYFFLSHLAFADMAYSSSVTPNMLVNFLVERNTVSYLGCAIQ
Tm7       GNVLVCMAVSREKALQTTTNYLIVSLAVADLLVATLVMPWVVYLEVVGEWKFSRIHCDIF

          ....|....|....|....|....|....|....|....|....|....|....|....|
GPCR4       IGSAAFFATVECVLLAAMAYDRFVAICSPLLYSTKMS----------------------
Tm7         VTLDVMMCTASILNLCAISIDRYTAVAMPMLYNTRYSSKRRVTVMIAIVWVLSFTISCPM

gnl|Pfam|pfam00001, 7tm_1, 7 transmembrane receptor (rhodopsin family)
Length = 377
             Score = 38.9 bits (89), Expect = 4e-04
                  310       320       330       340       350       360
           ....|....|....|....|....|....|....|....|....|....|....|....|
GPCR       ---------------------TILKMRSTEGHHKAFSTCTSHLTVVTLFYGTITFIYVMP
Tm7        NGHAKTVNPKIAKIFEIQSMPNGKTRTSLKTMSRRKLSQQKEKKATQMLAIVLGVFIICW

                   370       380       390       400
           ....|....|....|....|....|....|....|....|....
GPC        NFSMSITDQN-KVVSVLYTVVIPMLNPLIY
Tm7        LPFFIITHILNIHCDCN----IPPVLYSAFTWLGYVNSAVNPIIY
```

The nucleic acids and proteins of GPCR4 are useful in potential therapeutic applications implicated in various in various GPCR-related pathological disorders and/or OR-related pathological disorders, described further below. For example, a cDNA encoding the GPCR-or olfactory-receptor-like protein may be useful in gene therapy, and the receptor -like protein may be useful when administered to a subject in need thereof. By way of nonlimiting example, the compositions of the present invention will have efficacy for treatment of patients suffering from neoplasm, adenocarcinoma, lymphoma, prostate cancer, uterus cancer, immune response, AIDS, asthma, Crohn's disease, multiple sclerosis, and Albright Hereditary Ostoeodystrophy. Other GPCR-related diseases and disorders are contemplated.

The novel GPCR4 nucleic acids and proteins, or fragments thereof, may further be useful in diagnostic applications, wherein the presence or amount of the nucleic acid or the protein are to be assessed. These materials are further useful in the generation of antibodies that bind immunospecifically to the novel substances of the invention for use in therapeutic or

diagnostic methods. These antibodies may be generated according to methods known in the art, using prediction from hydrophobicity charts, as described in the "Anti-GPCRX Antibodies" section below. In one embodiment, a contemplated GPCR4 epitope is from about amino acids 10 to 30. In another embodiment, a GPCR4 epitope is from about amino acids 240 to 255. In additional embodiments, GPCR4 epitopes are from amino acids 310 to 330 and from amino acids 330 to 340. This novel protein also has value in development of powerful assay system for functional analysis of various human disorders, which will help in understanding of pathology of the disease and development of new drug targets for various disorders

GPCR5

The disclosed novel GPCR5 nucleic acid of 976 nucleotides (also referred to as AC021427_F) is shown in Table 5a. An open reading begins with an ATG initiation codon at nucleotides 23-25 and ends with a TAG codon at nucleotides 944-948. A putative untranslated region upstream from the initiation codon and downstream from the termination codon are underlined in Table 5a, and the start and stop codons are in bold letters.

Table 5A. GPCR5 Nucleotide Sequence (SEQ ID NO:16)

TCAACAGGAACAATTCACAAAG**ATG**GAGATTGGAAACCATACCACAGTGACAGAGTTTATTATTTTGG
GGTTAACTGAGGATCCTACACTTTGTGACATCTTCTTTGTGATATTTCTAGGAATCTACATTGTCACC
TTAATAGGCAATATCAGCATAATAAGAAGCTGTTCCCAACTTCACACTCCCATGTACCTGTTCCTCAG
CCACTTGGCTTTTGTGGACATAGGGCTTGCCACAGTAGTCACACCTATAATGCTTATGGGATTCCTAA
GACGTGGAACAGCCCTCCCTGTCACTAGCTGTGAAGCCCAGCTCTGTTCTGTAGTCATGTTTGGGACG
TCTGAATGCTTCCTACTGGCGACCATGGCCTATGATCGCTATGTGGCCATCTGCTCACCCCTGGTGAA
CTCCACCCACTTGTCCCCCATAATCTGCATACTCTTAGTGGGGGTTTGCTACCTGGGTGGATGTGTGA
ATGCCTCAACATTTACTAGTTGTTTATTGAGTCTGTCTTTCTGTGGACCAAATCAGATAGATCATTTT
TTCTGTGATTTCTCTCCTTTGTTGAAACTTTCCTGCTCAAATATCTCCATTCCTGAAATTATCCCTTC
CATCTCTTCTGGATCTATCATTGTGGTCACAGTATTTGCCATAGCCATCTCCTACATCTACATCCTCA
TCACCATCCTGAAGATGCGCTCCGCCGAGGGGCGCCACAAGGCCTTCTCCACCTGTACCTCCCACCTC
GCTGCGGTTACTCTCTACTATGGAACGATTACCTTCATTTATGTGATGCCCAAATCCAGTTACTCAAC
TAGCCAGAACAGATTGATATCGCTGTCCTACACAGTGGTAATCCCCATACTGAACCCCTTTATCTATA
GTCTGAGGAACAGAGATGTAAAGGAGGCACTAAGAAAGGCAACTGTCAGAATATATTCT**TAG**GATCAA
TTTGTAGCATTTCAGACGACCTAT

The GPCR5 protein encoded by SEQ ID NO:16 has 307 amino acid residues, and is presented using the one-letter code in Table 5B (SEQ ID NO:17). The SignalP, Psort and/or Hydropathy profile for GPCR5 predict that GPCR5 has a signal peptide and is likely to be localized at the plasma membrane with a certainty of 0.6000. The signalP shows a signal sequence is coded for in the first 48 amino acids, *i.e.*, with a cleavage site at the slash in the

sequence IRS-CS, between amino acids 44 and 45. This is typical of this type of membrane protein.

Table 5B. Encoded GPCR5 protein sequence (SEQ ID NO:17).

```
MEIGNHTTVTEFIILGLTEDPTLCDIFFVIFLGIYIVTLIGNISIIRS/CSQLHTPMYLFLSHLAFVD
IGLATVVTPIMLMGFLRRGTALPVTSCEAQLCSVVMFGTSECFLLATMAYDRYVAICSPLVNSTHLSP
IICILLVGVCYLGGCVNASTFTSCLLSLSFCGPNQIDHFFCDFSPLLKLSCSNISIPEIIPSISSGSI
IVVTVFAIAISYIYILITILKMRSAEGRHKAFSTCTSHLAAVTLYYGTITFIYVMPKSSYSTSQNRLI
SLSYTVVIPILNPFIYSLRNRDVKEALRKATVRIYS
```

The disclosed nucleic acid sequence has 648 of 899 bases (72%) identical to a *Mus musculus* odorant mRNA S25 gene (GENBANK-ID: AF121977) (E value = $7.5e\text{-}^{90}$).

The full amino acid sequence of the protein of the invention was found to have 216 of 311 amino acid residues (69%) identical to, and 261 of 311 residues (83%) positive with, the 342 amino acid residue odorant receptor protein from *Mus musculus* (ptnr:SPTREMBL-ACC:Q9WU91) (E value = $1.4e\text{-}^{112}$). BLAST analysis of the Patp database produced the significant results listed in Table 5C.

Table 5C Patp blast

Sequences producing High-scoring Segment Pairs:	Reading Frame	High Score	Smallest Sum Prob P(N)	N
patp:Y90876 Human G protein-coupled receptor GTAR11-2 ...	+2	703	1.4e-68	1
patp:Y90876 Human G protein-coupled receptor GTAR11-2 ...	+2	703	1.4e-68	1
patp:Y83389 Olfactory receptor protein OLF-4 - Homo sa...	+2	702	1.8e-68	1
patp:Y90877 Human G protein-coupled receptor GTAR11-3 ...	+2	700	2.9e-68	1

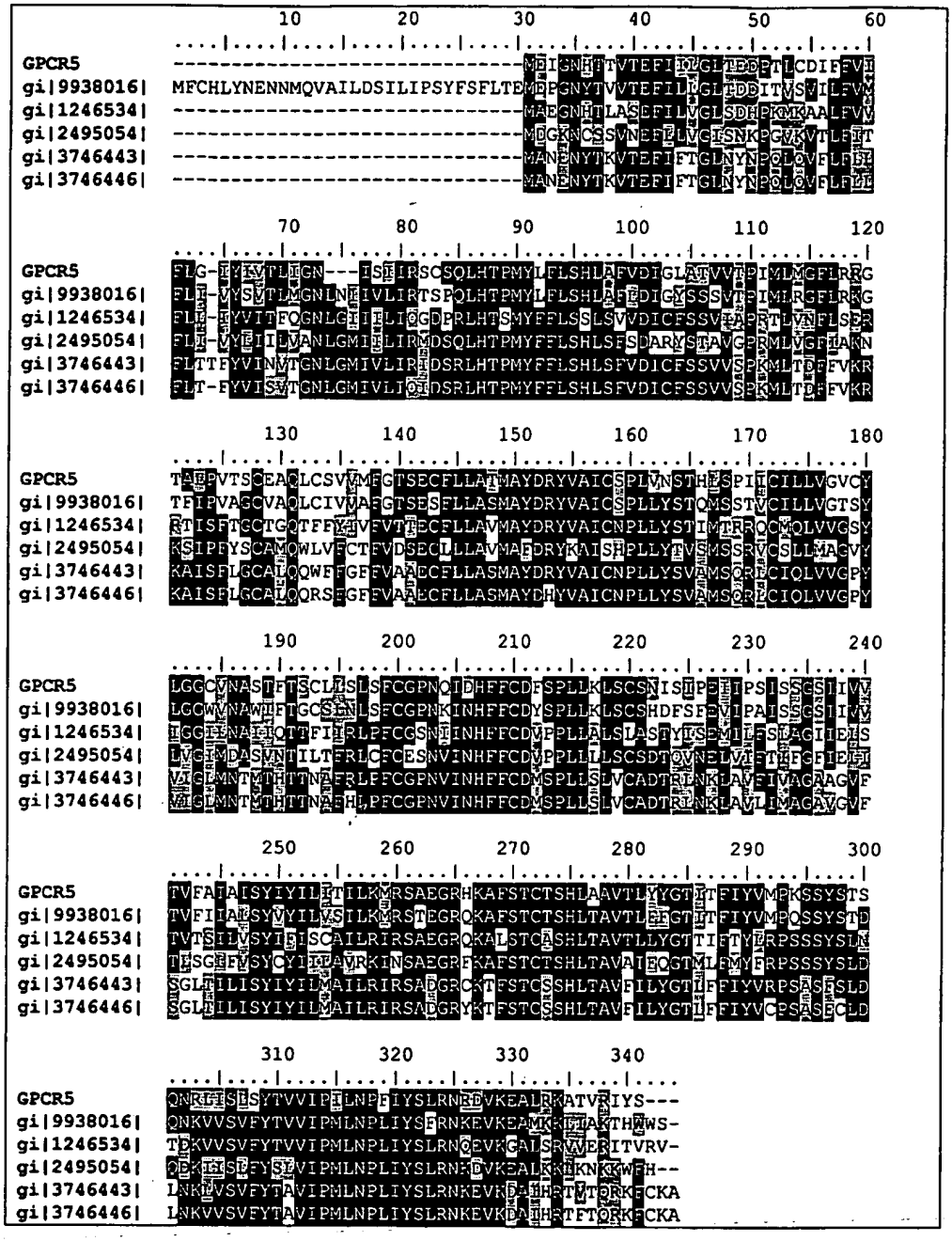
For example, the GPCR5 amino acid sequence has 137 of 303 amino acid residues (45 %) identical to, and 202 of 303 residues (66 %) positive with, the 313 amino acid residue G-protein coupled receptor from *Homo sapiens* (ppat: Y90876, PCT publication WO 00/21999) ($E= 1.4e^{-68}$).

A multiple sequence alignment is given in Table 5D, with GPCR5 being shown on line 1, in a ClustalW analysis comparing GPCR5 with related protein sequences.

Table 5D. Information for the ClustalW proteins:

1) GPCR5 (SEQ ID NO:17)
2) gi|9938016|ref|NP_064687.1| odorant receptor S25 gene [Mus musculus] (SEQ ID NO:25)
3) gi|1246534|emb|CAA64370.1| olfactory receptor 4 [Gallus gallus] (SEQ ID NO:21)
4) gi|2495054|sp|Q95155|OLF2_CANFA OLFACTORY RECEPTOR-LIKE PROTEIN OLF2 (SEQ ID NO:37)
5) gi|3746443|gb|AAC63969.1| olfactory receptor OR93Ch [Pan troglodytes] (SEQ ID NO:23)

6) gi|3746446|gb|AAC63970.1| olfactory receptor OR93Oo [Pongo pygmaeus] (SEQ ID NO:36)



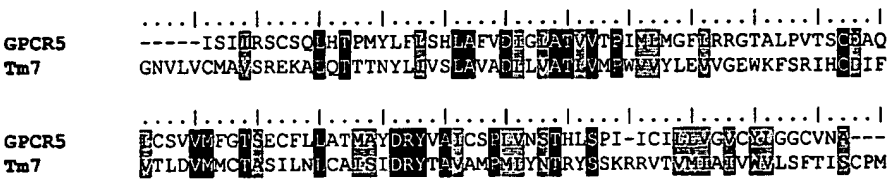
The presence of identifiable domains in GPCR5 was determined by searches using algorithms such as PROSITE, Blocks, Pfam, ProDomain, Prints and then determining the Interpro number by crossing the domain match (or numbers) using the Interpro website (http:www.ebi.ac.uk/interpro/).

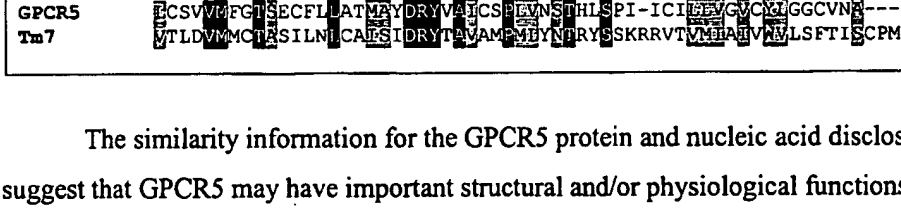
DOMAIN results for GPCR5 were collected from the Conserved Domain Database (CDD) with Reverse Position Specific BLAST. This BLAST samples domains found in the

Smart and Pfam collections. The results are listed in Table 5E with the statistics and domain description. The results indicate that this protein contains the following protein domains (as defined by Interpro) at the indicated positions: domain name 7tm_1 (InterPro) 7 transmembrane receptor (rhodopsin family) at amino acid positions 43 to 153. This indicates that the sequence of GPCR5 has properties similar to those of other proteins known to contain this/these domain(s) and similar to the properties of these domains.

Table 5E. DOMAIN results for GPCR5

gnl|Pfam|pfam00001, 7tm_1, 7 transmembrane receptor (rhodopsin family)
Length = 377 (SEQ ID NO:24).
Score = 68.6 bits (166), Expect = 5e-13





The similarity information for the GPCR5 protein and nucleic acid disclosed herein suggest that GPCR5 may have important structural and/or physiological functions characteristic of the Olfactory Receptor family and the GPCR family. Therefore, the nucleic acids and proteins of the invention are useful in potential diagnostic and therapeutic applications and as a research tool. These include serving as a specific or selective nucleic acid or protein diagnostic and/or prognostic marker, wherein the presence or amount of the nucleic acid or the protein are to be assessed, as well as potential therapeutic applications such as the following: (i) a protein therapeutic, (ii) a small molecule drug target, (iii) an antibody target (therapeutic, diagnostic, drug targeting/cytotoxic antibody), (iv) a nucleic acid useful in gene therapy (gene delivery/gene ablation), and (v) a composition promoting tissue regeneration in vitro and in vivo (vi) biological defense weapon. The novel nucleic acid encoding GPCR5, and the GPCR5 protein of the invention, or fragments thereof, may further be useful in diagnostic applications, wherein the presence or amount of the nucleic acid or the protein are to be assessed.

These materials are further useful in the generation of antibodies that bind immunospecifically to the novel GPCR5 substances for use in therapeutic or diagnostic methods. These antibodies may be generated according to methods known in the art, using prediction from hydrophobicity charts, as described in the "Anti-GPCRX Antibodies" section below. In one embodiment, a contemplated GPCR5 epitope is from about aa 220 to 240. In another

embodiment, a GPCR5 epitope is from about aa 250 to 260. In additional embodiments, GPCR1 epitopes are from aa 280 to 300.

A summary of the GPCRX nucleic acids and proteins of the invention is provided in Table 6A.

T

TABLE 6: Summary Of Nucleic Acids And Proteins Of The Invention

Name	Tables	Clone; Description of Homolog	Nucleic Acid SEQ ID NO	Amino Acid SEQ ID NO
GPCR1	1A, 1B	AC021427_A; AC021427_E; GPCR-like protein	1	2
GPCR2	2A, 2B, 2D, 2E, 2F, 2G, 2H, 2J, 2K	AC021427_B, AC021427_B1, AC021427_B_da1, AC021427_B_da2, AC021427_B2; AC021427_B_da3; GPCR-like protein	3, 5, 6, 8, 9, 10	4, 7, 11
GPCR3	3A, 3B	AC021427_C; GPCR-like protein	12	13
GPCR4	4A, 4B	AC021427_D; GPCR-like protein	14	15
GPCR5	5A, 5B	AC021427_F; GPCR-like protein	16	17

GPCRX Nucleic Acids and Polypeptides

One aspect of the invention pertains to isolated nucleic acid molecules that encode GPCRX polypeptides or biologically active portions thereof. Also included in the invention are nucleic acid fragments sufficient for use as hybridization probes to identify GPCRX-encoding nucleic acids (*e.g.*, GPCRX mRNAs) and fragments for use as PCR primers for the amplification and/or mutation of GPCRX nucleic acid molecules. As used herein, the term "nucleic acid molecule" is intended to include DNA molecules (*e.g.*, cDNA or genomic DNA), RNA molecules (*e.g.*, mRNA), analogs of the DNA or RNA generated using nucleotide analogs, and derivatives, fragments and homologs thereof. The nucleic acid molecule may be single-stranded or double-stranded, but preferably is comprised double-stranded DNA.

An GPCRX nucleic acid can encode a mature GPCRX polypeptide. As used herein, a "mature" form of a polypeptide or protein disclosed in the present invention is the product of a naturally occurring polypeptide or precursor form or proprotein. The naturally occurring polypeptide, precursor or proprotein includes, by way of nonlimiting example, the full-length

gene product, encoded by the corresponding gene. Alternatively, it may be defined as the polypeptide, precursor or proprotein encoded by an ORF described herein. The product "mature" form arises, again by way of nonlimiting example, as a result of one or more naturally occurring processing steps as they may take place within the cell, or host cell, in which the gene product arises. Examples of such processing steps leading to a "mature" form of a polypeptide or protein include the cleavage of the N-terminal methionine residue encoded by the initiation codon of an ORF, or the proteolytic cleavage of a signal peptide or leader sequence. Thus a mature form arising from a precursor polypeptide or protein that has residues 1 to N, where residue 1 is the N-terminal methionine, would have residues 2 through N remaining after removal of the N-terminal methionine. Alternatively, a mature form arising from a precursor polypeptide or protein having residues 1 to N, in which an N-terminal signal sequence from residue 1 to residue M is cleaved, would have the residues from residue M+1 to residue N remaining. Further as used herein, a "mature" form of a polypeptide or protein may arise from a step of post-translational modification other than a proteolytic cleavage event. Such additional processes include, by way of non-limiting example, glycosylation, myristoylation or phosphorylation. In general, a mature polypeptide or protein may result from the operation of only one of these processes, or a combination of any of them.

The term "probes", as utilized herein, refers to nucleic acid sequences of variable length, preferably between at least about 10 nucleotides (nt), 100 nt, or as many as approximately, *e.g.*, 6,000 nt, depending upon the specific use. Probes are used in the detection of identical, similar, or complementary nucleic acid sequences. Longer length probes are generally obtained from a natural or recombinant source, are highly specific, and much slower to hybridize than shorter-length oligomer probes. Probes may be single- or double-stranded and designed to have specificity in PCR, membrane-based hybridization technologies, or ELISA-like technologies.

The term "isolated" nucleic acid molecule, as utilized herein, is one, which is separated from other nucleic acid molecules which are present in the natural source of the nucleic acid. Preferably, an "isolated" nucleic acid is free of sequences which naturally flank the nucleic acid (*i.e.*, sequences located at the 5'- and 3'-termini of the nucleic acid) in the genomic DNA of the organism from which the nucleic acid is derived. For example, in various embodiments, the isolated GPCRX nucleic acid molecules can contain less than about 5 kb, 4 kb, 3 kb, 2 kb, 1 kb, 0.5 kb or 0.1 kb of nucleotide sequences which naturally flank the nucleic acid molecule in genomic DNA of the cell/tissue from which the nucleic acid is derived (*e.g.*, brain, heart, liver, spleen, etc.). Moreover, an "isolated" nucleic acid molecule, such as a cDNA molecule,

can be substantially free of other cellular material or culture medium when produced by recombinant techniques, or of chemical precursors or other chemicals when chemically synthesized.

A nucleic acid molecule of the invention, *e.g.*, a nucleic acid molecule having the nucleotide sequence of SEQ ID NOS:1, 3, 5, 6, 8, 9, 10, 12, 14, and 16, or a complement of this aforementioned nucleotide sequence, can be isolated using standard molecular biology techniques and the sequence information provided herein. Using all or a portion of the nucleic acid sequence of SEQ ID NOS:1, 3, 5, 6, 8, 9, 10, 12, 14, and 16 as a hybridization probe, GPCRX molecules can be isolated using standard hybridization and cloning techniques (*e.g.*, as described in Sambrook, *et al.*, (eds.), MOLECULAR CLONING: A LABORATORY MANUAL 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989; and Ausubel, *et al.*, (eds.), CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, New York, NY, 1993.)

A nucleic acid of the invention can be amplified using cDNA, mRNA or alternatively, genomic DNA, as a template and appropriate oligonucleotide primers according to standard PCR amplification techniques. The nucleic acid so amplified can be cloned into an appropriate vector and characterized by DNA sequence analysis. Furthermore, oligonucleotides corresponding to GPCRX nucleotide sequences can be prepared by standard synthetic techniques, *e.g.*, using an automated DNA synthesizer.

As used herein, the term "oligonucleotide" refers to a series of linked nucleotide residues, which oligonucleotide has a sufficient number of nucleotide bases to be used in a PCR reaction. A short oligonucleotide sequence may be based on, or designed from, a genomic or cDNA sequence and is used to amplify, confirm, or reveal the presence of an identical, similar or complementary DNA or RNA in a particular cell or tissue. Oligonucleotides comprise portions of a nucleic acid sequence having about 10 nt, 50 nt, or 100 nt in length, preferably about 15 nt to 30 nt in length. In one embodiment of the invention, an oligonucleotide comprising a nucleic acid molecule less than 100 nt in length would further comprise at least 6 contiguous nucleotides of SEQ ID NOS:1, 3, 5, 6, 8, 9, 10, 12, 14, and 16, or a complement thereof. Oligonucleotides may be chemically synthesized and may also be used as probes.

In another embodiment, an isolated nucleic acid molecule of the invention comprises a nucleic acid molecule that is a complement of the nucleotide sequence shown in SEQ ID NOS:1, 3, 5, 6, 8, 9, 10, 12, 14, and 16, or a portion of this nucleotide sequence (*e.g.*, a fragment that can be used as a probe or primer or a fragment encoding a biologically-active

portion of an GPCRX polypeptide). A nucleic acid molecule that is complementary to the nucleotide sequence shown in SEQ ID NOS:1, 3, 5, 6, 8, 9, 10, 12, 14, and 16, is one that is sufficiently complementary to the nucleotide sequence shown in SEQ ID NOS:1, 3, 5, 6, 8, 9, 10, 12, 14, and 16, that it can hydrogen bond with little or no mismatches to the nucleotide sequence shown SEQ ID NOS:1, 3, 5, 6, 8, 9, 10, 12, 14, and 16, thereby forming a stable duplex.

As used herein, the term "complementary" refers to Watson-Crick or Hoogsteen base pairing between nucleotides units of a nucleic acid molecule, and the term "binding" means the physical or chemical interaction between two polypeptides or compounds or associated polypeptides or compounds or combinations thereof. Binding includes ionic, non-ionic, van der Waals, hydrophobic interactions, and the like. A physical interaction can be either direct or indirect. Indirect interactions may be through or due to the effects of another polypeptide or compound. Direct binding refers to interactions that do not take place through, or due to, the effect of another polypeptide or compound, but instead are without other substantial chemical intermediates.

Fragments provided herein are defined as sequences of at least 6 (contiguous) nucleic acids or at least 4 (contiguous) amino acids, a length sufficient to allow for specific hybridization in the case of nucleic acids or for specific recognition of an epitope in the case of amino acids, respectively, and are at most some portion less than a full length sequence. Fragments may be derived from any contiguous portion of a nucleic acid or amino acid sequence of choice. Derivatives are nucleic acid sequences or amino acid sequences formed from the native compounds either directly or by modification or partial substitution. Analogs are nucleic acid sequences or amino acid sequences that have a structure similar to, but not identical to, the native compound but differs from it in respect to certain components or side chains. Analogs may be synthetic or from a different evolutionary origin and may have a similar or opposite metabolic activity compared to wild type. Homologs are nucleic acid sequences or amino acid sequences of a particular gene that are derived from different species.

Derivatives and analogs may be full length or other than full length, if the derivative or analog contains a modified nucleic acid or amino acid, as described below. Derivatives or analogs of the nucleic acids or proteins of the invention include, but are not limited to, molecules comprising regions that are substantially homologous to the nucleic acids or proteins of the invention, in various embodiments, by at least about 70%, 80%, or 95% identity (with a preferred identity of 80-95%) over a nucleic acid or amino acid sequence of identical size or when compared to an aligned sequence in which the alignment is done by a

computer homology program known in the art, or whose encoding nucleic acid is capable of hybridizing to the complement of a sequence encoding the aforementioned proteins under stringent, moderately stringent, or low stringent conditions. See *e.g.* Ausubel, *et al.*, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, New York, NY, 1993, and below.

A "homologous nucleic acid sequence" or "homologous amino acid sequence," or variations thereof, refer to sequences characterized by a homology at the nucleotide level or amino acid level as discussed above. Homologous nucleotide sequences encode those sequences coding for isoforms of GPCRX polypeptides. Isoforms can be expressed in different tissues of the same organism as a result of, for example, alternative splicing of RNA. Alternatively, isoforms can be encoded by different genes. In the invention, homologous nucleotide sequences include nucleotide sequences encoding for an GPCRX polypeptide of species other than humans, including, but not limited to: vertebrates, and thus can include, *e.g.*, frog, mouse, rat, rabbit, dog, cat cow, horse, and other organisms. Homologous nucleotide sequences also include, but are not limited to, naturally occurring allelic variations and mutations of the nucleotide sequences set forth herein. A homologous nucleotide sequence does not, however, include the exact nucleotide sequence encoding human GPCRX protein. Homologous nucleic acid sequences include those nucleic acid sequences that encode conservative amino acid substitutions (see below) in SEQ ID NOS:1, 3, 5, 6, 8, 9, 10, 12, 14, and 16, as well as a polypeptide possessing GPCRX biological activity. Various biological activities of the GPCRX proteins are described below.

An GPCRX polypeptide is encoded by the open reading frame ("ORF") of an GPCRX nucleic acid. An ORF corresponds to a nucleotide sequence that could potentially be translated into a polypeptide. A stretch of nucleic acids comprising an ORF is uninterrupted by a stop codon. An ORF that represents the coding sequence for a full protein begins with an ATG "start" codon and terminates with one of the three "stop" codons, namely, TAA, TAG, or TGA. For the purposes of this invention, an ORF may be any part of a coding sequence, with or without a start codon, a stop codon, or both. For an ORF to be considered as a good candidate for coding for a *bona fide* cellular protein, a minimum size requirement is often set, *e.g.*, a stretch of DNA that would encode a protein of 50 amino acids or more.

The nucleotide sequences determined from the cloning of the human GPCRX genes allows for the generation of probes and primers designed for use in identifying and/or cloning GPCRX homologues in other cell types, *e.g.* from other tissues, as well as GPCRX homologues from other vertebrates. The probe/primer typically comprises substantially purified oligonucleotide. The oligonucleotide typically comprises a region of nucleotide

sequence that hybridizes under stringent conditions to at least about 12, 25, 50, 100, 150, 200, 250, 300, 350 or 400 consecutive sense strand nucleotide sequence of SEQ ID NOS:1, 3, 5, 6, 8, 9, 10, 12, 14, and 16; or an anti-sense strand nucleotide sequence of SEQ ID NOS:1, 3, 5, 6, 8, 9, 10, 12, 14, and 16; or of a naturally occurring mutant of SEQ ID NOS:1, 3, 5, 6, 8, 9, 10, 12, 14, and 16.

Probes based on the human GPCRX nucleotide sequences can be used to detect transcripts or genomic sequences encoding the same or homologous proteins. In various embodiments, the probe further comprises a label group attached thereto, *e.g.* the label group can be a radioisotope, a fluorescent compound, an enzyme, or an enzyme co-factor. Such probes can be used as a part of a diagnostic test kit for identifying cells or tissues which mis-express an GPCRX protein, such as by measuring a level of an GPCRX-encoding nucleic acid in a sample of cells from a subject *e.g.*, detecting GPCRX mRNA levels or determining whether a genomic GPCRX gene has been mutated or deleted.

"A polypeptide having a biologically-active portion of an GPCRX polypeptide" refers to polypeptides exhibiting activity similar, but not necessarily identical to, an activity of a polypeptide of the invention, including mature forms, as measured in a particular biological assay, with or without dose dependency. A nucleic acid fragment encoding a "biologically-active portion of GPCRX" can be prepared by isolating a portion SEQ ID NOS:1, 3, 5, 6, 8, 9, 10, 12, 14, and 16 that encodes a polypeptide having an GPCRX biological activity (the biological activities of the GPCRX proteins are described below), expressing the encoded portion of GPCRX protein (*e.g.*, by recombinant expression *in vitro*) and assessing the activity of the encoded portion of GPCRX.

GPCRX Nucleic Acid and Polypeptide Variants

The invention further encompasses nucleic acid molecules that differ from the nucleotide sequences shown in SEQ ID NOS:1, 3, 5, 6, 8, 9, 10, 12, 14, and 16 due to degeneracy of the genetic code and thus encode the same GPCRX proteins as that encoded by the nucleotide sequences shown in SEQ ID NOS:1, 3, 5, 6, 8, 9, 10, 12, 14, and 16. In another embodiment, an isolated nucleic acid molecule of the invention has a nucleotide sequence encoding a protein having an amino acid sequence shown in SEQ ID NOS:2, 4, 7, 11, 13, 15, and 17.

In addition to the human GPCRX nucleotide sequences shown in SEQ ID NOS:1, 3, 5, 6, 8, 9, 10, 12, 14, and 16 it will be appreciated by those skilled in the art that DNA sequence polymorphisms that lead to changes in the amino acid sequences of the GPCRX polypeptides

may exist within a population (*e.g.*, the human population). Such genetic polymorphism in the GPCRX genes may exist among individuals within a population due to natural allelic variation. As used herein, the terms "gene" and "recombinant gene" refer to nucleic acid molecules comprising an open reading frame (ORF) encoding an GPCRX protein, preferably a vertebrate GPCRX protein. Such natural allelic variations can typically result in 1-5% variance in the nucleotide sequence of the GPCRX genes. Any and all such nucleotide variations and resulting amino acid polymorphisms in the GPCRX polypeptides, which are the result of natural allelic variation and that do not alter the functional activity of the GPCRX polypeptides, are intended to be within the scope of the invention.

Moreover, nucleic acid molecules encoding GPCRX proteins from other species, and thus that have a nucleotide sequence that differs from the human sequence of SEQ ID NOS:1, 3, 5, 6, 8, 9, 10, 12, 14, and 16 are intended to be within the scope of the invention. Nucleic acid molecules corresponding to natural allelic variants and homologues of the GPCRX cDNAs of the invention can be isolated based on their homology to the human GPCRX nucleic acids disclosed herein using the human cDNAs, or a portion thereof, as a hybridization probe according to standard hybridization techniques under stringent hybridization conditions.

Accordingly, in another embodiment, an isolated nucleic acid molecule of the invention is at least 6 nucleotides in length and hybridizes under stringent conditions to the nucleic acid molecule comprising the nucleotide sequence of SEQ ID NOS:1, 3, 5, 6, 8, 9, 10, 12, 14, and 16. In another embodiment, the nucleic acid is at least 10, 25, 50, 100, 250, 500, 750, 1000, 1500, or 2000 or more nucleotides in length. In yet another embodiment, an isolated nucleic acid molecule of the invention hybridizes to the coding region. As used herein, the term "hybridizes under stringent conditions" is intended to describe conditions for hybridization and washing under which nucleotide sequences at least 60% homologous to each other typically remain hybridized to each other.

Homologs (*i.e.*, nucleic acids encoding GPCRX proteins derived from species other than human) or other related sequences (*e.g.*, paralogs) can be obtained by low, moderate or high stringency hybridization with all or a portion of the particular human sequence as a probe using methods well known in the art for nucleic acid hybridization and cloning.

As used herein, the phrase "stringent hybridization conditions" refers to conditions under which a probe, primer or oligonucleotide will hybridize to its target sequence, but to no other sequences. Stringent conditions are sequence-dependent and will be different in different circumstances. Longer sequences hybridize specifically at higher temperatures than shorter sequences. Generally, stringent conditions are selected to be about 5 °C lower than the

thermal melting point (Tm) for the specific sequence at a defined ionic strength and pH. The Tm is the temperature (under defined ionic strength, pH and nucleic acid concentration) at which 50% of the probes complementary to the target sequence hybridize to the target sequence at equilibrium. Since the target sequences are generally present at excess, at Tm, 50% of the probes are occupied at equilibrium. Typically, stringent conditions will be those in which the salt concentration is less than about 1.0 M sodium ion, typically about 0.01 to 1.0 M sodium ion (or other salts) at

pH 7.0 to 8.3 and the temperature is at least about 30°C for short probes, primers or oligonucleotides (*e.g.*, 10 nt to 50 nt) and at least about 60°C for longer probes, primers and oligonucleotides. Stringent conditions may also be achieved with the addition of destabilizing agents, such as formamide.

Stringent conditions are known to those skilled in the art and can be found in Ausubel, *et al.*, (eds.), CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. Preferably, the conditions are such that sequences at least about 65%, 70%, 75%, 85%, 90%, 95%, 98%, or 99% homologous to each other typically remain hybridized to each other. A non-limiting example of stringent hybridization conditions are hybridization in a high salt buffer comprising 6X SSC, 50 mM Tris-HCl (pH 7.5), 1 mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.02% BSA, and 500 mg/ml denatured salmon sperm DNA at 65°C, followed by one or more washes in 0.2X SSC, 0.01% BSA at 50°C. An isolated nucleic acid molecule of the invention that hybridizes under stringent conditions to the sequences of SEQ ID NOS:1, 3, 5, 6, 8, 9, 10, 12, 14, and 16 corresponds to a naturally-occurring nucleic acid molecule. As used herein, a "naturally-occurring" nucleic acid molecule refers to an RNA or DNA molecule having a nucleotide sequence that occurs in nature (*e.g.*, encodes a natural protein).

In a second embodiment, a nucleic acid sequence that is hybridizable to the nucleic acid molecule comprising the nucleotide sequence of SEQ ID NOS:1, 3, 5, 6, 8, 9, 10, 12, 14, and 16 or fragments, analogs or derivatives thereof, under conditions of moderate stringency is provided. A non-limiting example of moderate stringency hybridization conditions are hybridization in 6X SSC, 5X Denhardt's solution, 0.5% SDS and 100 mg/ml denatured salmon sperm DNA at 55°C, followed by one or more washes in 1X SSC, 0.1% SDS at 37°C. Other conditions of moderate stringency that may be used are well-known within the art. See, *e.g.*, Ausubel, et *al.* (eds.), 1993, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, NY, and Kriegler, 1990; GENE TRANSFER AND EXPRESSION, A LABORATORY MANUAL, Stockton Press, NY.

In a third embodiment, a nucleic acid that is hybridizable to the nucleic acid molecule comprising the nucleotide sequences of SEQ ID NOS:1, 3, 5, 6, 8, 9, 10, 12, 14, and 16 or fragments, analogs or derivatives thereof, under conditions of low stringency, is provided. A non-limiting example of low stringency hybridization conditions are hybridization in 35% formamide, 5X SSC, 50 mM Tris-HCl (pH 7.5), 5 mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.2% BSA, 100 mg/ml denatured salmon sperm DNA, 10% (wt/vol) dextran sulfate at 40°C, followed by one or more washes in 2X SSC, 25 mM Tris-HCl (pH 7.4), 5 mM EDTA, and 0.1% SDS at 50°C. Other conditions of low stringency that may be used are well known in the art (*e.g.*, as employed for cross-species hybridizations). See, *e.g.*, Ausubel, *et al.* (eds.), 1993, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, NY, and Kriegler, 1990, GENE TRANSFER AND EXPRESSION, A LABORATORY MANUAL, Stockton Press, NY; Shilo and Weinberg, 1981. *Proc Natl Acad Sci USA* **78**: 6789-6792.

Conservative Mutations

In addition to naturally-occurring allelic variants of GPCRX sequences that may exist in the population, the skilled artisan will further appreciate that changes can be introduced by mutation into the nucleotide sequences of SEQ ID NOS:1, 3, 5, 6, 8, 9, 10, 12, 14, and 16 thereby leading to changes in the amino acid sequences of the encoded GPCRX proteins, without altering the functional ability of said GPCRX proteins. For example, nucleotide substitutions leading to amino acid substitutions at "non-essential" amino acid residues can be made in the sequence of SEQ ID NOS:2, 4, 7, 11, 13, 15, and 17. A "non-essential" amino acid residue is a residue that can be altered from the wild-type sequences of the GPCRX proteins without altering their biological activity, whereas an "essential" amino acid residue is required for such biological activity. For example, amino acid residues that are conserved among the GPCRX proteins of the invention are predicted to be particularly non-amenable to alteration. Amino acids for which conservative substitutions can be made are well-known within the art.

Another aspect of the invention pertains to nucleic acid molecules encoding GPCRX proteins that contain changes in amino acid residues that are not essential for activity. Such GPCRX proteins differ in amino acid sequence from SEQ ID NOS:2, 4, 7, 11, 13, 15, and 17 yet retain biological activity. In one embodiment, the isolated nucleic acid molecule comprises a nucleotide sequence encoding a protein, wherein the protein comprises an amino acid sequence at least about 45% homologous to the amino acid sequences of SEQ ID NOS:2, 4, 7, 11, 13, 15, and 17. Preferably, the protein encoded by the nucleic acid molecule is at

least about 60% homologous to SEQ ID NOS:2, 4, 7, 11, 13, 15, and 17; more preferably at least about 70% homologous to SEQ ID NOS:2, 4, 7, 11, 13, 15, and 17; still more preferably at least about 80% homologous to SEQ ID NOS:2, 4, 7, 11, 13, 15, and 17; even more preferably at least about 90% homologous to SEQ ID NOS:2, 4, 7, 11, 13, 15, and 17; and most preferably at least about 95% homologous to SEQ ID NOS:2, 4, 7, 11, 13, 15, and 17.

An isolated nucleic acid molecule encoding an GPCRX protein homologous to the protein of SEQ ID NOS:2, 4, 7, 11, 13, 15, and 17 can be created by introducing one or more nucleotide substitutions, additions or deletions into the nucleotide sequence of SEQ ID NOS:1, 3, 5, 6, 8, 9, 10, 12, 14, and 16 such that one or more amino acid substitutions, additions or deletions are introduced into the encoded protein.

Mutations can be introduced into SEQ ID NOS:2, 4, 7, 11, 13, 15, and 17 by standard techniques, such as site-directed mutagenesis and PCR-mediated mutagenesis. Preferably, conservative amino acid substitutions are made at one or more predicted, non-essential amino acid residues. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined within the art. These families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). Thus, a predicted non-essential amino acid residue in the GPCRX protein is replaced with another amino acid residue from the same side chain family. Alternatively, in another embodiment, mutations can be introduced randomly along all or part of an GPCRX coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for GPCRX biological activity to identify mutants that retain activity. Following mutagenesis of SEQ ID NOS:1, 3, 5, 6, 8, 9, 10, 12, 14 and 16, the encoded protein can be expressed by any recombinant technology known in the art and the activity of the protein can be determined.

The relatedness of amino acid families may also be determined based on side chain interactions. Substituted amino acids may be fully conserved "strong" residues or fully conserved "weak" residues. The "strong" group of conserved amino acid residues may be any one of the following groups: STA, NEQK, NHQK, NDEQ, QHRK, MILV, MILF, HY, FYW, wherein the single letter amino acid codes are grouped by those amino acids that may be

substituted for each other. Likewise, the "weak" group of conserved residues may be any one of the following: CSA, ATV, SAG, STNK, STPA, SGND, SNDEQK, NDEQHK, NEQHRK, VLIM, HFY, wherein the letters within each group represent the single letter amino acid code.

In one embodiment, a mutant GPCRX protein can be assayed for (*i*) the ability to form protein:protein interactions with other GPCRX proteins, other cell-surface proteins, or biologically-active portions thereof, (*ii*) complex formation between a mutant GPCRX protein and an GPCRX ligand; or (*iii*) the ability of a mutant GPCRX protein to bind to an intracellular target protein or biologically-active portion thereof; (*e.g.* avidin proteins).

In yet another embodiment, a mutant GPCRX protein can be assayed for the ability to regulate a specific biological function (*e.g.*, regulation of insulin release).

Antisense Nucleic Acids

Another aspect of the invention pertains to isolated antisense nucleic acid molecules that are hybridizable to or complementary to the nucleic acid molecule comprising the nucleotide sequence of SEQ ID NOS:1, 3, 5, 6, 8, 9, 10, 12, 14, and 16, or fragments, analogs or derivatives thereof. An "antisense" nucleic acid comprises a nucleotide sequence that is complementary to a "sense" nucleic acid encoding a protein (*e.g.*, complementary to the coding strand of a double-stranded cDNA molecule or complementary to an mRNA sequence). In specific aspects, antisense nucleic acid molecules are provided that comprise a sequence complementary to at least about 10, 25, 50, 100, 250 or 500 nucleotides or an entire GPCRX coding strand, or to only a portion thereof. Nucleic acid molecules encoding fragments, homologs, derivatives and analogs of an GPCRX protein of SEQ ID NOS:2, 4, 7, 11, 13, 15, and 17, or antisense nucleic acids complementary to an GPCRX nucleic acid sequence of SEQ ID NOS:1, 3, 5, 6, 8, 9, 10, 12, 14 and 16, are additionally provided.

In one embodiment, an antisense nucleic acid molecule is antisense to a "coding region" of the coding strand of a nucleotide sequence encoding an GPCRX protein. The term "coding region" refers to the region of the nucleotide sequence comprising codons which are translated into amino acid residues. In another embodiment, the antisense nucleic acid molecule is antisense to a "noncoding region" of the coding strand of a nucleotide sequence encoding the GPCRX protein. The term "noncoding region" refers to 5' and 3' sequences which flank the coding region that are not translated into amino acids (*i.e.*, also referred to as 5' and 3' untranslated regions).

Given the coding strand sequences encoding the GPCRX protein disclosed herein, antisense nucleic acids of the invention can be designed according to the rules of Watson and

Crick or Hoogsteen base pairing. The antisense nucleic acid molecule can be complementary to the entire coding region of GPCRX mRNA, but more preferably is an oligonucleotide that is antisense to only a portion of the coding or noncoding region of GPCRX mRNA. For example, the antisense oligonucleotide can be complementary to the region surrounding the translation start site of GPCRX mRNA. An antisense oligonucleotide can be, for example, about 5, 10, 15, 20, 25, 30, 35, 40, 45 or 50 nucleotides in length. An antisense nucleic acid of the invention can be constructed using chemical synthesis or enzymatic ligation reactions using procedures known in the art. For example, an antisense nucleic acid (*e.g.*, an antisense oligonucleotide) can be chemically synthesized using naturally-occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the antisense and sense nucleic acids (*e.g.*, phosphorothioate derivatives and acridine substituted nucleotides can be used).

Examples of modified nucleotides that can be used to generate the antisense nucleic acid include: 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxylmethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine. Alternatively, the antisense nucleic acid can be produced biologically using an expression vector into which a nucleic acid has been subcloned in an antisense orientation (*i.e.*, RNA transcribed from the inserted nucleic acid will be of an antisense orientation to a target nucleic acid of interest, described further in the following subsection).

The antisense nucleic acid molecules of the invention are typically administered to a subject or generated *in situ* such that they hybridize with or bind to cellular mRNA and/or genomic DNA encoding an GPCRX protein to thereby inhibit expression of the protein (*e.g.*, by inhibiting transcription and/or translation). The hybridization can be by conventional nucleotide complementarity to form a stable duplex, or, for example, in the case of an antisense nucleic acid molecule that binds to DNA duplexes, through specific interactions in

the major groove of the double helix. An example of a route of administration of antisense nucleic acid molecules of the invention includes direct injection at a tissue site. Alternatively, antisense nucleic acid molecules can be modified to target selected cells and then administered systemically. For example, for systemic administration, antisense molecules can be modified such that they specifically bind to receptors or antigens expressed on a selected cell surface (*e.g.*, by linking the antisense nucleic acid molecules to peptides or antibodies that bind to cell surface receptors or antigens). The antisense nucleic acid molecules can also be delivered to cells using the vectors described herein. To achieve sufficient nucleic acid molecules, vector constructs in which the antisense nucleic acid molecule is placed under the control of a strong pol II or pol III promoter are preferred.

In yet another embodiment, the antisense nucleic acid molecule of the invention is an α-anomeric nucleic acid molecule. An α-anomeric nucleic acid molecule forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual β-units, the strands run parallel to each other. See, *e.g.*, Gaultier, *et al.*, 1987. *Nucl. Acids Res.* **15**: 6625-6641. The antisense nucleic acid molecule can also comprise a 2'-o-methylribonucleotide (see, *e.g.*, Inoue, *et al.* 1987. *Nucl. Acids Res.* **15**: 6131-6148) or a chimeric RNA-DNA analogue (see, *e.g.*, Inoue, *et al.*, 1987. *FEBS Lett.* **215**: 327-330.

Ribozymes and PNA Moieties

Nucleic acid modifications include, by way of non-limiting example, modified bases, and nucleic acids whose sugar phosphate backbones are modified or derivatized. These modifications are carried out at least in part to enhance the chemical stability of the modified nucleic acid, such that they may be used, for example, as antisense binding nucleic acids in therapeutic applications in a subject.

In one embodiment, an antisense nucleic acid of the invention is a ribozyme. Ribozymes are catalytic RNA molecules with ribonuclease activity that are capable of cleaving a single-stranded nucleic acid, such as an mRNA, to which they have a complementary region. Thus, ribozymes (*e.g.*, hammerhead ribozymes as described in Haselhoff and Gerlach 1988. *Nature* 334: 585-591) can be used to catalytically cleave GPCRX mRNA transcripts to thereby inhibit translation of GPCRX mRNA. A ribozyme having specificity for an GPCRX-encoding nucleic acid can be designed based upon the nucleotide sequence of an GPCRX cDNA disclosed herein (*i.e.*, SEQ ID NOS:1, 3, 5, 6, 8, 9, 10, 12, 14, and 16). For example, a derivative of a *Tetrahymena* L-19 IVS RNA can be constructed in which the nucleotide sequence of the active site is complementary to the nucleotide sequence

to be cleaved in an GPCRX-encoding mRNA. See, *e.g.*, U.S. Patent 4,987,071 to Cech, *et al.* and U.S. Patent 5,116,742 to Cech, *et al.* GPCRX mRNA can also be used to select a catalytic RNA having a specific ribonuclease activity from a pool of RNA molecules. See, *e.g.*, Bartel *et al.*, (1993) *Science* 261:1411-1418.

Alternatively, GPCRX gene expression can be inhibited by targeting nucleotide sequences complementary to the regulatory region of the GPCRX nucleic acid (*e.g.*, the GPCRX promoter and/or enhancers) to form triple helical structures that prevent transcription of the GPCRX gene in target cells. *See, e.g.,* Helene, 1991. *Anticancer Drug Des.* 6: 569-84; Helene, *et al.* 1992. *Ann. N.Y. Acad. Sci.* 660: 27-36; Maher, 1992. *Bioassays* 14: 807-15.

In various embodiments, the GPCRX nucleic acids can be modified at the base moiety, sugar moiety or phosphate backbone to improve, *e.g.*, the stability, hybridization, or solubility of the molecule. For example, the deoxyribose phosphate backbone of the nucleic acids can be modified to generate peptide nucleic acids. *See, e.g.,* Hyrup, *et al.*, 1996. *Bioorg Med Chem* 4: 5-23. As used herein, the terms "peptide nucleic acids" or "PNAs" refer to nucleic acid mimics (*e.g.*, DNA mimics) in which the deoxyribose phosphate backbone is replaced by a pseudopeptide backbone and only the four natural nucleobases are retained. The neutral backbone of PNAs has been shown to allow for specific hybridization to DNA and RNA under conditions of low ionic strength. The synthesis of PNA oligomers can be performed using standard solid phase peptide synthesis protocols as described in Hyrup, *et al.*, 1996. *supra*; Perry-O'Keefe, *et al.*, 1996. *Proc. Natl. Acad. Sci. USA* 93: 14670-14675.

PNAs of GPCRX can be used in therapeutic and diagnostic applications. For example, PNAs can be used as antisense or antigene agents for sequence-specific modulation of gene expression by, *e.g.*, inducing transcription or translation arrest or inhibiting replication. PNAs of GPCRX can also be used, for example, in the analysis of single base pair mutations in a gene (*e.g.*, PNA directed PCR clamping; as artificial restriction enzymes when used in combination with other enzymes, *e.g.*, S_1 nucleases (*see,* Hyrup, *et al.*, 1996.*supra*); or as probes or primers for DNA sequence and hybridization (*see,* Hyrup, *et al.*, 1996, *supra*; Perry-O'Keefe, *et al.*, 1996. *supra*).

In another embodiment, PNAs of GPCRX can be modified, *e.g.*, to enhance their stability or cellular uptake, by attaching lipophilic or other helper groups to PNA, by the formation of PNA-DNA chimeras, or by the use of liposomes or other techniques of drug delivery known in the art. For example, PNA-DNA chimeras of GPCRX can be generated that may combine the advantageous properties of PNA and DNA. Such chimeras allow DNA recognition enzymes (*e.g.*, RNase H and DNA polymerases) to interact with the DNA portion

while the PNA portion would provide high binding affinity and specificity. PNA-DNA chimeras can be linked using linkers of appropriate lengths selected in terms of base stacking, number of bonds between the nucleobases, and orientation (*see*, Hyrup, et al., 1996. *supra*). The synthesis of PNA-DNA chimeras can be performed as described in Hyrup, *et al.*, 1996. *supra* and Finn, *et al.*, 1996. *Nucl Acids Res* 24: 3357-3363. For example, a DNA chain can be synthesized on a solid support using standard phosphoramidite coupling chemistry, and modified nucleoside analogs, *e.g.*, 5'-(4-methoxytrityl)amino-5'-deoxy-thymidine phosphoramidite, can be used between the PNA and the 5' end of DNA. *See, e.g.*, Mag, *et al.*, 1989. *Nucl Acid Res* 17: 5973-5988. PNA monomers are then coupled in a stepwise manner to produce a chimeric molecule with a 5' PNA segment and a 3' DNA segment. *See, e.g.*, Finn, *et al.*, 1996. *supra*. Alternatively, chimeric molecules can be synthesized with a 5' DNA segment and a 3' PNA segment. *See, e.g.*, Petersen, *et al.*, 1975. *Bioorg. Med. Chem. Lett.* 5: 1119-11124.

In other embodiments, the oligonucleotide may include other appended groups such as peptides (*e.g.*, for targeting host cell receptors *in vivo*), or agents facilitating transport across the cell membrane (*see, e.g.*, Letsinger, *et al.*, 1989. *Proc. Natl. Acad. Sci. U.S.A.* 86: 6553-6556; Lemaitre, *et al.*, 1987. *Proc. Natl. Acad. Sci.* 84: 648-652; PCT Publication No. WO88/09810) or the blood-brain barrier (*see, e.g.*, PCT Publication No. WO 89/10134). In addition, oligonucleotides can be modified with hybridization triggered cleavage agents (*see, e.g.*, Krol, *et al.*, 1988. *BioTechniques* 6:958-976) or intercalating agents (*see, e.g.*, Zon, 1988. *Pharm. Res.* 5: 539-549). To this end, the oligonucleotide may be conjugated to another molecule, *e.g.*, a peptide, a hybridization triggered cross-linking agent, a transport agent, a hybridization-triggered cleavage agent, and the like.

GPCRX Polypeptides

A polypeptide according to the invention includes a polypeptide including the amino acid sequence of GPCRX polypeptides whose sequences are provided in SEQ ID NOS:2, 4, 7, 11, 13, 15, and 17. The invention also includes a mutant or variant protein any of whose residues may be changed from the corresponding residues shown in SEQ ID NOS:2, 4, 7, 11, 13, 15, and 17 while still encoding a protein that maintains its GPCRX activities and physiological functions, or a functional fragment thereof.

In general, an GPCRX variant that preserves GPCRX-like function includes any variant in which residues at a particular position in the sequence have been substituted by other amino acids, and further include the possibility of inserting an additional residue or

residues between two residues of the parent protein as well as the possibility of deleting one or more residues from the parent sequence. Any amino acid substitution, insertion, or deletion is encompassed by the invention. In favorable circumstances, the substitution is a conservative substitution as defined above.

One aspect of the invention pertains to isolated GPCRX proteins, and biologically-active portions thereof, or derivatives, fragments, analogs or homologs thereof. Also provided are polypeptide fragments suitable for use as immunogens to raise anti-GPCRX antibodies. In one embodiment, native GPCRX proteins can be isolated from cells or tissue sources by an appropriate purification scheme using standard protein purification techniques. In another embodiment, GPCRX proteins are produced by recombinant DNA techniques. Alternative to recombinant expression, an GPCRX protein or polypeptide can be synthesized chemically using standard peptide synthesis techniques.

An "isolated" or "purified" polypeptide or protein or biologically-active portion thereof is substantially free of cellular material or other contaminating proteins from the cell or tissue source from which the GPCRX protein is derived, or substantially free from chemical precursors or other chemicals when chemically synthesized. The language "substantially free of cellular material" includes preparations of GPCRX proteins in which the protein is separated from cellular components of the cells from which it is isolated or recombinantly-produced. In one embodiment, the language "substantially free of cellular material" includes preparations of GPCRX proteins having less than about 30% (by dry weight) of non-GPCRX proteins (also referred to herein as a "contaminating protein"), more preferably less than about 20% of non-GPCRX proteins, still more preferably less than about 10% of non-GPCRX proteins, and most preferably less than about 5% of non-GPCRX proteins. When the GPCRX protein or biologically-active portion thereof is recombinantly-produced, it is also preferably substantially free of culture medium, *i.e.*, culture medium represents less than about 20%, more preferably less than about 10%, and most preferably less than about 5% of the volume of the GPCRX protein preparation.

The language "substantially free of chemical precursors or other chemicals" includes preparations of GPCRX proteins in which the protein is separated from chemical precursors or other chemicals that are involved in the synthesis of the protein. In one embodiment, the language "substantially free of chemical precursors or other chemicals" includes preparations of GPCRX proteins having less than about 30% (by dry weight) of chemical precursors or non-GPCRX chemicals, more preferably less than about 20% chemical precursors or non-GPCRX chemicals, still more preferably less than about 10% chemical precursors or

non-GPCRX chemicals, and most preferably less than about 5% chemical precursors or non-GPCRX chemicals.

Biologically-active portions of GPCRX proteins include peptides comprising amino acid sequences sufficiently homologous to or derived from the amino acid sequences of the GPCRX proteins (*e.g.*, the amino acid sequence shown in SEQ ID NOS:2, 4, 7, 11, 13, 15, and 17) that include fewer amino acids than the full-length GPCRX proteins, and exhibit at least one activity of an GPCRX protein. Typically, biologically-active portions comprise a domain or motif with at least one activity of the GPCRX protein. A biologically-active portion of an GPCRX protein can be a polypeptide which is, for example, 10, 25, 50, 100 or more amino acid residues in length.

Moreover, other biologically-active portions, in which other regions of the protein are deleted, can be prepared by recombinant techniques and evaluated for one or more of the functional activities of a native GPCRX protein.

In an embodiment, the GPCRX protein has an amino acid sequence shown in SEQ ID NOS:2, 4, 7, 11, 13, 15, and 17. In other embodiments, the GPCRX protein is substantially homologous to SEQ ID NOS:2, 4, 7, 11, 13, 15, and 17, and retains the functional activity of the protein of SEQ ID NOS:2, 4, 7, 11, 13, 15, and 17, yet differs in amino acid sequence due to natural allelic variation or mutagenesis, as described in detail, below. Accordingly, in another embodiment, the GPCRX protein is a protein that comprises an amino acid sequence at least about 45% homologous to the amino acid sequence of SEQ ID NOS:2, 4, 7, 11, 13, 15, and 17, and retains the functional activity of the GPCRX proteins of SEQ ID NOS:2, 4, 7, 11, 13, 15, and 17.

Determining Homology Between Two or More Sequences

To determine the percent homology of two amino acid sequences or of two nucleic acids, the sequences are aligned for optimal comparison purposes (*e.g.*, gaps can be introduced in the sequence of a first amino acid or nucleic acid sequence for optimal alignment with a second amino or nucleic acid sequence). The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are homologous at that position (*i.e.*, as used herein amino acid or nucleic acid "homology" is equivalent to amino acid or nucleic acid "identity").

The nucleic acid sequence homology may be determined as the degree of identity between two sequences. The homology may be determined using computer programs known in the art, such as GAP software provided in the GCG program package. *See,* Needleman and Wunsch, 1970. *J Mol Biol* 48: 443-453. Using GCG GAP software with the following settings for nucleic acid sequence comparison: GAP creation penalty of 5.0 and GAP extension penalty of 0.3, the coding region of the analogous nucleic acid sequences referred to above exhibits a degree of identity preferably of at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99%, with the CDS (encoding) part of the DNA sequence shown in SEQ ID NOS:1, 3, 5, 6, 8, 9, 10, 12, 14, and 16.

The term "sequence identity" refers to the degree to which two polynucleotide or polypeptide sequences are identical on a residue-by-residue basis over a particular region of comparison. The term "percentage of sequence identity" is calculated by comparing two optimally aligned sequences over that region of comparison, determining the number of positions at which the identical nucleic acid base (*e.g.,* A, T, C, G, U, or I, in the case of nucleic acids) occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the region of comparison (*i.e.,* the window size), and multiplying the result by 100 to yield the percentage of sequence identity. The term "substantial identity" as used herein denotes a characteristic of a polynucleotide sequence, wherein the polynucleotide comprises a sequence that has at least 80 percent sequence identity, preferably at least 85 percent identity and often 90 to 95 percent sequence identity, more usually at least 99 percent sequence identity as compared to a reference sequence over a comparison region.

Chimeric and Fusion Proteins

The invention also provides GPCRX chimeric or fusion proteins. As used herein, an GPCRX "chimeric protein" or "fusion protein" comprises an GPCRX polypeptide operatively-linked to a non-GPCRX polypeptide. An "GPCRX polypeptide" refers to a polypeptide having an amino acid sequence corresponding to an GPCRX protein (SEQ ID NOS:2, 4, 7, 11, 13, 15, and 17), whereas a "non-GPCRX polypeptide" refers to a polypeptide having an amino acid sequence corresponding to a protein that is not substantially homologous to the GPCRX protein, *e.g.,* a protein that is different from the GPCRX protein and that is derived from the same or a different organism. Within an GPCRX fusion protein the GPCRX polypeptide can correspond to all or a portion of an GPCRX protein. In one embodiment, an GPCRX fusion protein comprises at least one biologically-active portion of an GPCRX protein. In another

embodiment, an GPCRX fusion protein comprises at least two biologically-active portions of an GPCRX protein. In yet another embodiment, an GPCRX fusion protein comprises at least three biologically-active portions of an GPCRX protein. Within the fusion protein, the term "operatively-linked" is intended to indicate that the GPCRX polypeptide and the non-GPCRX polypeptide are fused in-frame with one another. The non-GPCRX polypeptide can be fused to the N-terminus or C-terminus of the GPCRX polypeptide.

In one embodiment, the fusion protein is a GST-GPCRX fusion protein in which the GPCRX sequences are fused to the C-terminus of the GST (glutathione S-transferase) sequences. Such fusion proteins can facilitate the purification of recombinant GPCRX polypeptides.

In another embodiment, the fusion protein is an GPCRX protein containing a heterologous signal sequence at its N-terminus. In certain host cells (*e.g.*, mammalian host cells), expression and/or secretion of GPCRX can be increased through use of a heterologous signal sequence.

In yet another embodiment, the fusion protein is an GPCRX-immunoglobulin fusion protein in which the GPCRX sequences are fused to sequences derived from a member of the immunoglobulin protein family. The GPCRX-immunoglobulin fusion proteins of the invention can be incorporated into pharmaceutical compositions and administered to a subject to inhibit an interaction between an GPCRX ligand and an GPCRX protein on the surface of a cell, to thereby suppress GPCRX-mediated signal transduction *in vivo*. The GPCRX-immunoglobulin fusion proteins can be used to affect the bioavailability of an GPCRX cognate ligand. Inhibition of the GPCRX ligand/GPCRX interaction may be useful therapeutically for both the treatment of proliferative and differentiative disorders, as well as modulating (*e.g.* promoting or inhibiting) cell survival. Moreover, the GPCRX-immunoglobulin fusion proteins of the invention can be used as immunogens to produce anti-GPCRX antibodies in a subject, to purify GPCRX ligands, and in screening assays to identify molecules that inhibit the interaction of GPCRX with an GPCRX ligand.

An GPCRX chimeric or fusion protein of the invention can be produced by standard recombinant DNA techniques. For example, DNA fragments coding for the different polypeptide sequences are ligated together in-frame in accordance with conventional techniques, *e.g.*, by employing blunt-ended or stagger-ended termini for ligation, restriction enzyme digestion to provide for appropriate termini, filling-in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and enzymatic ligation. In another embodiment, the fusion gene can be synthesized by conventional techniques including

automated DNA synthesizers. Alternatively, PCR amplification of gene fragments can be carried out using anchor primers that give rise to complementary overhangs between two consecutive gene fragments that can subsequently be annealed and reamplified to generate a chimeric gene sequence (*see, e.g.,* Ausubel, *et al.* (eds.) CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, 1992). Moreover, many expression vectors are commercially available that already encode a fusion moiety (*e.g.*, a GST polypeptide). An GPCRX-encoding nucleic acid can be cloned into such an expression vector such that the fusion moiety is linked in-frame to the GPCRX protein.

GPCRX Agonists and Antagonists

The invention also pertains to variants of the GPCRX proteins that function as either GPCRX agonists (*i.e.,* mimetics) or as GPCRX antagonists. Variants of the GPCRX protein can be generated by mutagenesis (*e.g.*, discrete point mutation or truncation of the GPCRX protein). An agonist of the GPCRX protein can retain substantially the same, or a subset of, the biological activities of the naturally occurring form of the GPCRX protein. An antagonist of the GPCRX protein can inhibit one or more of the activities of the naturally occurring form of the GPCRX protein by, for example, competitively binding to a downstream or upstream member of a cellular signaling cascade which includes the GPCRX protein. Thus, specific biological effects can be elicited by treatment with a variant of limited function. In one embodiment, treatment of a subject with a variant having a subset of the biological activities of the naturally occurring form of the protein has fewer side effects in a subject relative to treatment with the naturally occurring form of the GPCRX proteins.

Variants of the GPCRX proteins that function as either GPCRX agonists (*i.e.,* mimetics) or as GPCRX antagonists can be identified by screening combinatorial libraries of mutants (*e.g.*, truncation mutants) of the GPCRX proteins for GPCRX protein agonist or antagonist activity. In one embodiment, a variegated library of GPCRX variants is generated by combinatorial mutagenesis at the nucleic acid level and is encoded by a variegated gene library. A variegated library of GPCRX variants can be produced by, for example, enzymatically ligating a mixture of synthetic oligonucleotides into gene sequences such that a degenerate set of potential GPCRX sequences is expressible as individual polypeptides, or alternatively, as a set of larger fusion proteins (*e.g.*, for phage display) containing the set of GPCRX sequences therein. There are a variety of methods which can be used to produce libraries of potential GPCRX variants from a degenerate oligonucleotide sequence. Chemical synthesis of a degenerate gene sequence can be performed in an automatic DNA synthesizer,

and the synthetic gene then ligated into an appropriate expression vector. Use of a degenerate set of genes allows for the provision, in one mixture, of all of the sequences encoding the desired set of potential GPCRX sequences. Methods for synthesizing degenerate oligonucleotides are well-known within the art. *See, e.g.,* Narang, 1983. *Tetrahedron* 39: 3; Itakura, *et al.*, 1984. *Annu. Rev. Biochem.* 53: 323; Itakura, *et al.*, 1984. *Science* 198: 1056; Ike, *et al.*, 1983. *Nucl. Acids Res.* 11: 477.

Polypeptide Libraries

In addition, libraries of fragments of the GPCRX protein coding sequences can be used to generate a variegated population of GPCRX fragments for screening and subsequent selection of variants of an GPCRX protein. In one embodiment, a library of coding sequence fragments can be generated by treating a double stranded PCR fragment of an GPCRX coding sequence with a nuclease under conditions wherein nicking occurs only about once per molecule, denaturing the double stranded DNA, renaturing the DNA to form double-stranded DNA that can include sense/antisense pairs from different nicked products, removing single stranded portions from reformed duplexes by treatment with S_1 nuclease, and ligating the resulting fragment library into an expression vector. By this method, expression libraries can be derived which encodes N-terminal and internal fragments of various sizes of the GPCRX proteins.

Various techniques are known in the art for screening gene products of combinatorial libraries made by point mutations or truncation, and for screening cDNA libraries for gene products having a selected property. Such techniques are adaptable for rapid screening of the gene libraries generated by the combinatorial mutagenesis of GPCRX proteins. The most widely used techniques, which are amenable to high throughput analysis, for screening large gene libraries typically include cloning the gene library into replicable expression vectors, transforming appropriate cells with the resulting library of vectors, and expressing the combinatorial genes under conditions in which detection of a desired activity facilitates isolation of the vector encoding the gene whose product was detected. Recursive ensemble mutagenesis (REM), a new technique that enhances the frequency of functional mutants in the libraries, can be used in combination with the screening assays to identify GPCRX variants. *See, e.g.,* Arkin and Yourvan, 1992. *Proc. Natl. Acad. Sci. USA* 89: 7811-7815; Delgrave, *et al.*, 1993. *Protein Engineering* 6:327-331.

Anti-GPCRX Antibodies

The invention encompasses antibodies and antibody fragments, such as F_{ab} or $(F_{ab})_2$, that bind immunospecifically to any of the GPCRX polypeptides of said invention.

An isolated GPCRX protein, or a portion or fragment thereof, can be used as an immunogen to generate antibodies that bind to GPCRX polypeptides using standard techniques for polyclonal and monoclonal antibody preparation. The full-length GPCRX proteins can be used or, alternatively, the invention provides antigenic peptide fragments of GPCRX proteins for use as immunogens. The antigenic GPCRX peptides comprises at least 4 amino acid residues of the amino acid sequence shown in SEQ ID NOS:2, 4, 7, 11, 13, 15, and 17 and encompasses an epitope of GPCRX such that an antibody raised against the peptide forms a specific immune complex with GPCRX. Preferably, the antigenic peptide comprises at least 6, 8, 10, 15, 20, or 30 amino acid residues. Longer antigenic peptides are sometimes preferable over shorter antigenic peptides, depending on use and according to methods well known to someone skilled in the art.

In certain embodiments of the invention, at least one epitope encompassed by the antigenic peptide is a region of GPCRX that is located on the surface of the protein (*e.g.*, a hydrophilic region). As a means for targeting antibody production, hydropathy plots showing regions of hydrophilicity and hydrophobicity may be generated by any method well known in the art, including, for example, the Kyte Doolittle or the Hopp Woods methods, either with or without Fourier transformation (*see, e.g.*, Hopp and Woods, 1981. *Proc. Nat. Acad. Sci. USA* 78: 3824-3828; Kyte and Doolittle, 1982. *J. Mol. Biol.* 157: 105-142, each incorporated herein by reference in their entirety).

As disclosed herein, GPCRX protein sequences of SEQ ID NOS:2, 4, 7, 11, 13, 15, and 17, or derivatives, fragments, analogs or homologs thereof, may be utilized as immunogens in the generation of antibodies that immunospecifically-bind these protein components. The term "antibody" as used herein refers to immunoglobulin molecules and immunologically-active portions of immunoglobulin molecules, *i.e.*, molecules that contain an antigen binding site that specifically-binds (immunoreacts with) an antigen, such as GPCRX. Such antibodies include, but are not limited to, polyclonal, monoclonal, chimeric, single chain, F_{ab} and $F_{(ab')2}$ fragments, and an F_{ab} expression library. In a specific embodiment, antibodies to human GPCRX proteins are disclosed. Various procedures known within the art may be used for the production of polyclonal or monoclonal antibodies to an GPCRX protein sequence of SEQ ID NOS:2, 4, 7, 11, 13, 15, and 17, or a derivative, fragment, analog or homolog thereof. Some of these proteins are discussed below.

For the production of polyclonal antibodies, various suitable host animals (*e.g.*, rabbit, goat, mouse or other mammal) may be immunized by injection with the native protein, or a synthetic variant thereof, or a derivative of the foregoing. An appropriate immunogenic preparation can contain, for example, recombinantly-expressed GPCRX protein or a chemically-synthesized GPCRX polypeptide. The preparation can further include an adjuvant. Various adjuvants used to increase the immunological response include, but are not limited to, Freund's (complete and incomplete), mineral gels (*e.g.*, aluminum hydroxide), surface active substances (*e.g.*, lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, dinitrophenol, etc.), human adjuvants such as *Bacille Calmette-Guerin* and *Corynebacterium parvum*, or similar immunostimulatory agents. If desired, the antibody molecules directed against GPCRX can be isolated from the mammal (*e.g.*, from the blood) and further purified by well known techniques, such as protein A chromatography to obtain the IgG fraction.

The term "monoclonal antibody" or "monoclonal antibody composition", as used herein, refers to a population of antibody molecules that contain only one species of an antigen binding site capable of immunoreacting with a particular epitope of GPCRX. A monoclonal antibody composition thus typically displays a single binding affinity for a particular GPCRX protein with which it immunoreacts. For preparation of monoclonal antibodies directed towards a particular GPCRX protein, or derivatives, fragments, analogs or homologs thereof, any technique that provides for the production of antibody molecules by continuous cell line culture may be utilized. Such techniques include, but are not limited to, the hybridoma technique (*see, e.g.*, Kohler & Milstein, 1975. *Nature* 256: 495-497); the trioma technique; the human B-cell hybridoma technique (*see, e.g.*, Kozbor, *et al.*, 1983. *Immunol. Today* 4: 72) and the EBV hybridoma technique to produce human monoclonal antibodies (*see, e.g.*, Cole, *et al.*, 1985. In: MONOCLONAL ANTIBODIES AND CANCER THERAPY, Alan R. Liss, Inc., pp. 77-96). Human monoclonal antibodies may be utilized in the practice of the invention and may be produced by using human hybridomas (*see, e.g.*, Cote, *et al.*, 1983. *Proc Natl Acad Sci USA* 80: 2026-2030) or by transforming human B-cells with Epstein Barr Virus *in vitro* (*see, e.g.*, Cole, *et al.*, 1985. In: MONOCLONAL ANTIBODIES AND CANCER THERAPY, Alan R. Liss, Inc., pp. 77-96). Each of the above citations is incorporated herein by reference in their entirety.

According to the invention, techniques can be adapted for the production of single-chain antibodies specific to an GPCRX protein (*see, e.g.*, U.S. Patent No. 4,946,778). In addition, methods can be adapted for the construction of F_{ab} expression libraries (*see, e.g.*, Huse, *et al.*, 1989. *Science* 246: 1275-1281) to allow rapid and effective identification of monoclonal F_{ab} fragments with the desired specificity for an GPCRX protein or derivatives,

fragments, analogs or homologs thereof. Non-human antibodies can be "humanized" by techniques well known in the art. *See, e.g.,* U.S. Patent No. 5,225,539. Antibody fragments that contain the idiotypes to an GPCRX protein may be produced by techniques known in the art including, but not limited to: (*i*) an $F_{(ab')2}$ fragment produced by pepsin digestion of an antibody molecule; (*ii*) an F_{ab} fragment generated by reducing the disulfide bridges of an $F_{(ab')2}$ fragment; (*iii*) an F_{ab} fragment generated by the treatment of the antibody molecule with papain and a reducing agent; and (*iv*) F_v fragments.

Additionally, recombinant anti-GPCRX antibodies, such as chimeric and humanized monoclonal antibodies, comprising both human and non-human portions, which can be made using standard recombinant DNA techniques, are within the scope of the invention. Such chimeric and humanized monoclonal antibodies can be produced by recombinant DNA techniques known in the art, for example using methods described in International Application No. PCT/US86/02269; European Patent Application No. 184,187; European Patent Application No. 171,496; European Patent Application No. 173,494; PCT International Publication No. WO 86/01533; U.S. Patent No. 4,816,567; U.S. Pat. No. 5,225,539; European Patent Application No. 125,023; Better, *et al.*, 1988. *Science* 240: 1041-1043; Liu, *et al.*, 1987. *Proc. Natl. Acad. Sci. USA* 84: 3439-3443; Liu, *et al.*, 1987. *J. Immunol.* 139: 3521-3526; Sun, *et al.*, 1987. *Proc. Natl. Acad. Sci. USA* 84: 214-218; Nishimura, *et al.*, 1987. *Cancer Res.* 47: 999-1005; Wood, *et al.*, 1985. *Nature* 314 :446-449; Shaw, *et al.*, 1988. *J. Natl. Cancer Inst.* 80: 1553-1559); Morrison(1985) *Science* 229:1202-1207; Oi, *et al.* (1986) *BioTechniques* 4:214; Jones, *et al.*, 1986. *Nature* 321: 552-525; Verhoeyan, *et al.*, 1988. *Science* 239: 1534; and Beidler, *et al.*, 1988. *J. Immunol.* 141: 4053-4060. Each of the above citations are incorporated herein by reference in their entirety.

In one embodiment, methods for the screening of antibodies that possess the desired specificity include, but are not limited to, enzyme-linked immunosorbent assay (ELISA) and other immunologically-mediated techniques known within the art. In a specific embodiment, selection of antibodies that are specific to a particular domain of an GPCRX protein is facilitated by generation of hybridomas that bind to the fragment of an GPCRX protein possessing such a domain. Thus, antibodies that are specific for a desired domain within an GPCRX protein, or derivatives, fragments, analogs or homologs thereof, are also provided herein.

Anti-GPCRX antibodies may be used in methods known within the art relating to the localization and/or quantitation of an GPCRX protein (*e.g.,* for use in measuring levels of the GPCRX protein within appropriate physiological samples, for use in diagnostic methods, for

use in imaging the protein, and the like). In a given embodiment, antibodies for GPCRX proteins, or derivatives, fragments, analogs or homologs thereof, that contain the antibody derived binding domain, are utilized as pharmacologically-active compounds (hereinafter "Therapeutics").

An anti-GPCRX antibody (*e.g.*, monoclonal antibody) can be used to isolate an GPCRX polypeptide by standard techniques, such as affinity chromatography or immunoprecipitation. An anti-GPCRX antibody can facilitate the purification of natural GPCRX polypeptide from cells and of recombinantly-produced GPCRX polypeptide expressed in host cells. Moreover, an anti-GPCRX antibody can be used to detect GPCRX protein (*e.g.*, in a cellular lysate or cell supernatant) in order to evaluate the abundance and pattern of expression of the GPCRX protein. Anti-GPCRX antibodies can be used diagnostically to monitor protein levels in tissue as part of a clinical testing procedure, *e.g.*, to, for example, determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling (*i.e.*, physically linking) the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin, and examples of suitable radioactive material include ^{125}I, ^{131}I, ^{35}S or ^{3}H.

GPCRX Recombinant Expression Vectors and Host Cells

Another aspect of the invention pertains to vectors, preferably expression vectors, containing a nucleic acid encoding an GPCRX protein, or derivatives, fragments, analogs or homologs thereof. As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments can be ligated. Another type of vector is a viral vector, wherein additional DNA segments can be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (*e.g.*, bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (*e.g.*,

non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively-linked. Such vectors are referred to herein as "expression vectors". In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" can be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors (*e.g.*, replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

The recombinant expression vectors of the invention comprise a nucleic acid of the invention in a form suitable for expression of the nucleic acid in a host cell, which means that the recombinant expression vectors include one or more regulatory sequences, selected on the basis of the host cells to be used for expression, that is operatively-linked to the nucleic acid sequence to be expressed. Within a recombinant expression vector, "operably-linked" is intended to mean that the nucleotide sequence of interest is linked to the regulatory sequence(s) in a manner that allows for expression of the nucleotide sequence (*e.g.*, in an *in vitro* transcription/translation system or in a host cell when the vector is introduced into the host cell).

The term "regulatory sequence" is intended to includes promoters, enhancers and other expression control elements (*e.g.*, polyadenylation signals). Such regulatory sequences are described, for example, in Goeddel, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990). Regulatory sequences include those that direct constitutive expression of a nucleotide sequence in many types of host cell and those that direct expression of the nucleotide sequence only in certain host cells (*e.g.*, tissue-specific regulatory sequences). It will be appreciated by those skilled in the art that the design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, etc. The expression vectors of the invention can be introduced into host cells to thereby produce proteins or peptides, including fusion proteins or peptides, encoded by nucleic acids as described herein (*e.g.*, GPCRX proteins, mutant forms of GPCRX proteins, fusion proteins, etc.).

The recombinant expression vectors of the invention can be designed for expression of GPCRX proteins in prokaryotic or eukaryotic cells. For example, GPCRX proteins can be expressed in bacterial cells such as *Escherichia coli*, insect cells (using baculovirus expression vectors) yeast cells or mammalian cells. Suitable host cells are discussed further in Goeddel,

GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990). Alternatively, the recombinant expression vector can be transcribed and translated *in vitro*, for example using T7 promoter regulatory sequences and T7 polymerase.

Expression of proteins in prokaryotes is most often carried out in *Escherichia coli* with vectors containing constitutive or inducible promoters directing the expression of either fusion or non-fusion proteins. Fusion vectors add a number of amino acids to a protein encoded therein, usually to the amino terminus of the recombinant protein. Such fusion vectors typically serve three purposes: (*i*) to increase expression of recombinant protein; (*ii*) to increase the solubility of the recombinant protein; and (*iii*) to aid in the purification of the recombinant protein by acting as a ligand in affinity purification. Often, in fusion expression vectors, a proteolytic cleavage site is introduced at the junction of the fusion moiety and the recombinant protein to enable separation of the recombinant protein from the fusion moiety subsequent to purification of the fusion protein. Such enzymes, and their cognate recognition sequences, include Factor Xa, thrombin and enterokinase. Typical fusion expression vectors include pGEX (Pharmacia Biotech Inc; Smith and Johnson, 1988. *Gene* 67: 31-40), pMAL (New England Biolabs, Beverly, Mass.) and pRIT5 (Pharmacia, Piscataway, N.J.) that fuse glutathione S-transferase (GST), maltose E binding protein, or protein A, respectively, to the target recombinant protein.

Examples of suitable inducible non-fusion *E. coli* expression vectors include pTrc (Amrann *et al.*, (1988) *Gene* 69:301-315) and pET 11d (Studier *et al.*, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990) 60-89).

One strategy to maximize recombinant protein expression in *E. coli* is to express the protein in a host bacteria with an impaired capacity to proteolytically cleave the recombinant protein. *See, e.g.*, Gottesman, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990) 119-128. Another strategy is to alter the nucleic acid sequence of the nucleic acid to be inserted into an expression vector so that the individual codons for each amino acid are those preferentially utilized in *E. coli* (*see, e.g.*, Wada, *et al.*, 1992. *Nucl. Acids Res.* 20: 2111-2118). Such alteration of nucleic acid sequences of the invention can be carried out by standard DNA synthesis techniques.

In another embodiment, the GPCRX expression vector is a yeast expression vector. Examples of vectors for expression in yeast *Saccharomyces cerivisae* include pYepSec1 (Baldari, *et al.*, 1987. *EMBO J.* 6: 229-234), pMFa (Kurjan and Herskowitz, 1982. *Cell* 30:

933-943), pJRY88 (Schultz *et al.*, 1987. *Gene* 54: 113-123), pYES2 (Invitrogen Corporation, San Diego, Calif.), and picZ (InVitrogen Corp, San Diego, Calif.).

Alternatively, GPCRX can be expressed in insect cells using baculovirus expression vectors. Baculovirus vectors available for expression of proteins in cultured insect cells (*e.g.*, SF9 cells) include the pAc series (Smith, *et al.*, 1983. *Mol. Cell. Biol.* 3: 2156-2165) and the pVL series (Lucklow and Summers, 1989. *Virology* 170: 31-39).

In yet another embodiment, a nucleic acid of the invention is expressed in mammalian cells using a mammalian expression vector. Examples of mammalian expression vectors include pCDM8 (Seed, 1987. *Nature* 329: 840) and pMT2PC (Kaufman, *et al.*, 1987. *EMBO J.* 6: 187-195). When used in mammalian cells, the expression vector's control functions are often provided by viral regulatory elements. For example, commonly used promoters are derived from polyoma, adenovirus 2, cytomegalovirus, and simian virus 40. For other suitable expression systems for both prokaryotic and eukaryotic cells see, *e.g.*, Chapters 16 and 17 of Sambrook, *et al.*, MOLECULAR CLONING: A LABORATORY MANUAL. 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989.

In another embodiment, the recombinant mammalian expression vector is capable of directing expression of the nucleic acid preferentially in a particular cell type (*e.g.*, tissue-specific regulatory elements are used to express the nucleic acid). Tissue-specific regulatory elements are known in the art. Non-limiting examples of suitable tissue-specific promoters include the albumin promoter (liver-specific; Pinkert, *et al.*, 1987. *Genes Dev.* 1: 268-277), lymphoid-specific promoters (Calame and Eaton, 1988. *Adv. Immunol.* 43: 235-275), in particular promoters of T cell receptors (Winoto and Baltimore, 1989. *EMBO J.* 8: 729-733) and immunoglobulins (Banerji, *et al.*, 1983. *Cell* 33: 729-740; Queen and Baltimore, 1983. *Cell* 33: 741-748), neuron-specific promoters (*e.g.*, the neurofilament promoter; Byrne and Ruddle, 1989. *Proc. Natl. Acad. Sci. USA* 86: 5473-5477), pancreas-specific promoters (Edlund, *et al.*, 1985. *Science* 230: 912-916), and mammary gland-specific promoters (*e.g.*, milk whey promoter; U.S. Pat. No. 4,873,316 and European Application Publication No. 264,166). Developmentally-regulated promoters are also encompassed, *e.g.*, the murine hox promoters (Kessel and Gruss, 1990. *Science* 249: 374-379) and the α-fetoprotein promoter (Campes and Tilghman, 1989. *Genes Dev.* 3: 537-546).

The invention further provides a recombinant expression vector comprising a DNA molecule of the invention cloned into the expression vector in an antisense orientation. That is, the DNA molecule is operatively-linked to a regulatory sequence in a manner that allows for expression (by transcription of the DNA molecule) of an RNA molecule that is antisense to

GPCRX mRNA. Regulatory sequences operatively linked to a nucleic acid cloned in the antisense orientation can be chosen that direct the continuous expression of the antisense RNA molecule in a variety of cell types, for instance viral promoters and/or enhancers, or regulatory sequences can be chosen that direct constitutive, tissue specific or cell type specific expression of antisense RNA. The antisense expression vector can be in the form of a recombinant plasmid, phagemid or attenuated virus in which antisense nucleic acids are produced under the control of a high efficiency regulatory region, the activity of which can be determined by the cell type into which the vector is introduced. For a discussion of the regulation of gene expression using antisense genes *see, e.g.*, Weintraub, *et al.*, "Antisense RNA as a molecular tool for genetic analysis," *Reviews-Trends in Genetics*, Vol. 1(1) 1986.

Another aspect of the invention pertains to host cells into which a recombinant expression vector of the invention has been introduced. The terms "host cell" and "recombinant host cell" are used interchangeably herein. It is understood that such terms refer not only to the particular subject cell but also to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

A host cell can be any prokaryotic or eukaryotic cell. For example, GPCRX protein can be expressed in bacterial cells such as *E. coli*, insect cells, yeast or mammalian cells (such as Chinese hamster ovary cells (CHO) or COS cells). Other suitable host cells are known to those skilled in the art.

Vector DNA can be introduced into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques. As used herein, the terms "transformation" and "transfection" are intended to refer to a variety of art-recognized techniques for introducing foreign nucleic acid (*e.g.*, DNA) into a host cell, including calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, or electroporation. Suitable methods for transforming or transfecting host cells can be found in Sambrook, *et al.* (MOLECULAR CLONING: A LABORATORY MANUAL. 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989), and other laboratory manuals.

For stable transfection of mammalian cells, it is known that, depending upon the expression vector and transfection technique used, only a small fraction of cells may integrate the foreign DNA into their genome. In order to identify and select these integrants, a gene that encodes a selectable marker (*e.g.*, resistance to antibiotics) is generally introduced into the

host cells along with the gene of interest. Various selectable markers include those that confer resistance to drugs, such as G418, hygromycin and methotrexate. Nucleic acid encoding a selectable marker can be introduced into a host cell on the same vector as that encoding GPCRX or can be introduced on a separate vector. Cells stably transfected with the introduced nucleic acid can be identified by drug selection (*e.g.*, cells that have incorporated the selectable marker gene will survive, while the other cells die).

A host cell of the invention, such as a prokaryotic or eukaryotic host cell in culture, can be used to produce (*i.e.*, express) GPCRX protein. Accordingly, the invention further provides methods for producing GPCRX protein using the host cells of the invention. In one embodiment, the method comprises culturing the host cell of invention (into which a recombinant expression vector encoding GPCRX protein has been introduced) in a suitable medium such that GPCRX protein is produced. In another embodiment, the method further comprises isolating GPCRX protein from the medium or the host cell.

Transgenic GPCRX Animals

The host cells of the invention can also be used to produce non-human transgenic animals. For example, in one embodiment, a host cell of the invention is a fertilized oocyte or an embryonic stem cell into which GPCRX protein-coding sequences have been introduced. Such host cells can then be used to create non-human transgenic animals in which exogenous GPCRX sequences have been introduced into their genome or homologous recombinant animals in which endogenous GPCRX sequences have been altered. Such animals are useful for studying the function and/or activity of GPCRX protein and for identifying and/or evaluating modulators of GPCRX protein activity. As used herein, a "transgenic animal" is a non-human animal, preferably a mammal, more preferably a rodent such as a rat or mouse, in which one or more of the cells of the animal includes a transgene. Other examples of transgenic animals include non-human primates, sheep, dogs, cows, goats, chickens, amphibians, etc. A transgene is exogenous DNA that is integrated into the genome of a cell from which a transgenic animal develops and that remains in the genome of the mature animal, thereby directing the expression of an encoded gene product in one or more cell types or tissues of the transgenic animal. As used herein, a "homologous recombinant animal" is a non-human animal, preferably a mammal, more preferably a mouse, in which an endogenous GPCRX gene has been altered by homologous recombination between the endogenous gene and an exogenous DNA molecule introduced into a cell of the animal, *e.g.*, an embryonic cell of the animal, prior to development of the animal.

A transgenic animal of the invention can be created by introducing GPCRX-encoding nucleic acid into the male pronuclei of a fertilized oocyte (*e.g.*, by microinjection, retroviral infection) and allowing the oocyte to develop in a pseudopregnant female foster animal. The human GPCRX cDNA sequences of SEQ ID NOS:1, 3, 5, 6, 8, 9, 10, 12, 14, and 16 can be introduced as a transgene into the genome of a non-human animal. Alternatively, a non-human homologue of the human GPCRX gene, such as a mouse GPCRX gene, can be isolated based on hybridization to the human GPCRX cDNA (described further *supra*) and used as a transgene. Intronic sequences and polyadenylation signals can also be included in the transgene to increase the efficiency of expression of the transgene. A tissue-specific regulatory sequence(s) can be operably-linked to the GPCRX transgene to direct expression of GPCRX protein to particular cells. Methods for generating transgenic animals via embryo manipulation and microinjection, particularly animals such as mice, have become conventional in the art and are described, for example, in U.S. Patent Nos. 4,736,866; 4,870,009; and 4,873,191; and Hogan, 1986. In: MANIPULATING THE MOUSE EMBRYO, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. Similar methods are used for production of other transgenic animals. A transgenic founder animal can be identified based upon the presence of the GPCRX transgene in its genome and/or expression of GPCRX mRNA in tissues or cells of the animals. A transgenic founder animal can then be used to breed additional animals carrying the transgene. Moreover, transgenic animals carrying a transgene-encoding GPCRX protein can further be bred to other transgenic animals carrying other transgenes.

To create a homologous recombinant animal, a vector is prepared which contains at least a portion of an GPCRX gene into which a deletion, addition or substitution has been introduced to thereby alter, *e.g.*, functionally disrupt, the GPCRX gene. The GPCRX gene can be a human gene (*e.g.*, the cDNA of SEQ ID NOS:1, 3, 5, 6, 8, 9, 10, 12, 14 and 16), but more preferably, is a non-human homologue of a human GPCRX gene. For example, a mouse homologue of human GPCRX gene of SEQ ID NOS:1, 3, 5, 6, 8, 9, 10, 12, 14, and 16 can be used to construct a homologous recombination vector suitable for altering an endogenous GPCRX gene in the mouse genome. In one embodiment, the vector is designed such that, upon homologous recombination, the endogenous GPCRX gene is functionally disrupted (*i.e.*, no longer encodes a functional protein; also referred to as a "knock out" vector).

Alternatively, the vector can be designed such that, upon homologous recombination, the endogenous GPCRX gene is mutated or otherwise altered but still encodes functional protein (*e.g.*, the upstream regulatory region can be altered to thereby alter the expression of

the endogenous GPCRX protein). In the homologous recombination vector, the altered portion of the GPCRX gene is flanked at its 5'- and 3'-termini by additional nucleic acid of the GPCRX gene to allow for homologous recombination to occur between the exogenous GPCRX gene carried by the vector and an endogenous GPCRX gene in an embryonic stem cell. The additional flanking GPCRX nucleic acid is of sufficient length for successful homologous recombination with the endogenous gene. Typically, several kilobases of flanking DNA (both at the 5'- and 3'-termini) are included in the vector. *See, e.g.,* Thomas, *et al.*, 1987. *Cell* 51: 503 for a description of homologous recombination vectors. The vector is ten introduced into an embryonic stem cell line (*e.g.*, by electroporation) and cells in which the introduced GPCRX gene has homologously-recombined with the endogenous GPCRX gene are selected. *See, e.g.*, Li, *et al.*, 1992. *Cell* 69: 915.

The selected cells are then injected into a blastocyst of an animal (*e.g.*, a mouse) to form aggregation chimeras. *See, e.g.*, Bradley, 1987. In: TERATOCARCINOMAS AND EMBRYONIC STEM CELLS: A PRACTICAL APPROACH, Robertson, ed. IRL, Oxford, pp. 113-152. A chimeric embryo can then be implanted into a suitable pseudopregnant female foster animal and the embryo brought to term. Progeny harboring the homologously-recombined DNA in their germ cells can be used to breed animals in which all cells of the animal contain the homologously-recombined DNA by germline transmission of the transgene. Methods for constructing homologous recombination vectors and homologous recombinant animals are described further in Bradley, 1991. *Curr. Opin. Biotechnol.* 2: 823-829; PCT International Publication Nos.: WO 90/11354; WO 91/01140; WO 92/0968; and WO 93/04169.

In another embodiment, transgenic non-humans animals can be produced that contain selected systems that allow for regulated expression of the transgene. One example of such a system is the cre/loxP recombinase system of bacteriophage P1. For a description of the cre/loxP recombinase system, *See, e.g.*, Lakso, *et al.*, 1992. *Proc. Natl. Acad. Sci. USA* 89: 6232-6236. Another example of a recombinase system is the FLP recombinase system of *Saccharomyces cerevisiae. See,* O'Gorman, *et al.*, 1991. *Science* 251:1351-1355. If a cre/loxP recombinase system is used to regulate expression of the transgene, animals containing transgenes encoding both the Cre recombinase and a selected protein are required. Such animals can be provided through the construction of "double" transgenic animals, *e.g.*, by mating two transgenic animals, one containing a transgene encoding a selected protein and the other containing a transgene encoding a recombinase.

Clones of the non-human transgenic animals described herein can also be produced according to the methods described in Wilmut, *et al.*, 1997. *Nature* 385: 810-813. In brief, a

cell (*e.g.*, a somatic cell) from the transgenic animal can be isolated and induced to exit the growth cycle and enter G_0 phase. The quiescent cell can then be fused, *e.g.*, through the use of electrical pulses, to an enucleated oocyte from an animal of the same species from which the quiescent cell is isolated. The reconstructed oocyte is then cultured such that it develops to morula or blastocyte and then transferred to pseudopregnant female foster animal. The offspring borne of this female foster animal will be a clone of the animal from which the cell (*e.g.*, the somatic cell) is isolated.

Pharmaceutical Compositions

The GPCRX nucleic acid molecules, GPCRX proteins, and anti-GPCRX antibodies (also referred to herein as "active compounds") of the invention, and derivatives, fragments, analogs and homologs thereof, can be incorporated into pharmaceutical compositions suitable for administration. Such compositions typically comprise the nucleic acid molecule, protein, or antibody and a pharmaceutically acceptable carrier. As used herein, "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. Suitable carriers are described in the most recent edition of Remington's Pharmaceutical Sciences, a standard reference text in the field, which is incorporated herein by reference. Preferred examples of such carriers or diluents include, but are not limited to, water, saline, finger's solutions, dextrose solution, and 5% human serum albumin. Liposomes and non-aqueous vehicles such as fixed oils may also be used. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, *e.g.*, intravenous, intradermal, subcutaneous, oral (*e.g.*, inhalation), transdermal (*i.e.*, topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid (EDTA); buffers such as acetates, citrates or phosphates,

and agents for the adjustment of tonicity such as sodium chloride or dextrose. The pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, N.J.) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringeability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as manitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions can be prepared by incorporating the active compound (*e.g.*, an GPCRX protein or anti-GPCRX antibody) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, methods of preparation are vacuum drying and freeze-drying that yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic

administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied orally and swished and expectorated or swallowed. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

For administration by inhalation, the compounds are delivered in the form of an aerosol spray from pressured container or dispenser which contains a suitable propellant, *e.g.*, a gas such as carbon dioxide, or a nebulizer.

Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

The compounds can also be prepared in the form of suppositories (*e.g.*, with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared

according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811.

It is especially advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and the limitations inherent in the art of compounding such an active compound for the treatment of individuals.

The nucleic acid molecules of the invention can be inserted into vectors and used as gene therapy vectors. Gene therapy vectors can be delivered to a subject by, for example, intravenous injection, local administration (*see, e.g.,* U.S. Patent No. 5,328,470) or by stereotactic injection (*see, e.g.*, Chen, *et al.*, 1994. *Proc. Natl. Acad. Sci. USA* 91: 3054-3057). The pharmaceutical preparation of the gene therapy vector can include the gene therapy vector in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery vector can be produced intact from recombinant cells, *e.g.*, retroviral vectors, the pharmaceutical preparation can include one or more cells that produce the gene delivery system.

The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

Screening and Detection Methods

The isolated nucleic acid molecules of the invention can be used to express GPCRX protein (*e.g.*, via a recombinant expression vector in a host cell in gene therapy applications), to detect GPCRX mRNA (*e.g.*, in a biological sample) or a genetic lesion in an GPCRX gene, and to modulate GPCRX activity, as described further, below. In addition, the GPCRX proteins can be used to screen drugs or compounds that modulate the GPCRX protein activity or expression as well as to treat disorders characterized by insufficient or excessive production of GPCRX protein or production of GPCRX protein forms that have decreased or aberrant activity compared to GPCRX wild-type protein (*e.g.*; diabetes (regulates insulin release); obesity (binds and transport lipids); metabolic disturbances associated with obesity, the metabolic syndrome X as well as anorexia and wasting disorders associated with chronic

diseases and various cancers, and infectious disease(possesses anti-microbial activity) and the various dyslipidemias. In addition, the anti-GPCRX antibodies of the invention can be used to detect and isolate GPCRX proteins and modulate GPCRX activity. In yet a further aspect, the invention can be used in methods to influence appetite, absorption of nutrients and the disposition of metabolic substrates in both a positive and negative fashion.

The invention further pertains to novel agents identified by the screening assays described herein and uses thereof for treatments as described, *supra*.

Screening Assays

The invention provides a method (also referred to herein as a "screening assay") for identifying modulators, *i.e.*, candidate or test compounds or agents (*e.g.*, peptides, peptidomimetics, small molecules or other drugs) that bind to GPCRX proteins or have a stimulatory or inhibitory effect on, *e.g.*, GPCRX protein expression or GPCRX protein activity. The invention also includes compounds identified in the screening assays described herein.

In one embodiment, the invention provides assays for screening candidate or test compounds which bind to or modulate the activity of the membrane-bound form of an GPCRX protein or polypeptide or biologically-active portion thereof. The test compounds of the invention can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including: biological libraries; spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; the "one-bead one-compound" library method; and synthetic library methods using affinity chromatography selection. The biological library approach is limited to peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer or small molecule libraries of compounds. *See, e.g.*, Lam, 1997. *Anticancer Drug Design* 12: 145.

A "small molecule" as used herein, is meant to refer to a composition that has a molecular weight of less than about 5 kD and most preferably less than about 4 kD. Small molecules can be, *e.g.*, nucleic acids, peptides, polypeptides, peptidomimetics, carbohydrates, lipids or other organic or inorganic molecules. Libraries of chemical and/or biological mixtures, such as fungal, bacterial, or algal extracts, are known in the art and can be screened with any of the assays of the invention.

Examples of methods for the synthesis of molecular libraries can be found in the art, for example in: DeWitt, *et al.*, 1993. *Proc. Natl. Acad. Sci. U.S.A.* 90: 6909; Erb, *et al.*, 1994. *Proc. Natl. Acad. Sci. U.S.A.* 91: 11422; Zuckermann, *et al.*, 1994. *J. Med. Chem.* 37: 2678;

Cho, *et al.*, 1993. *Science* 261: 1303; Carrell, *et al.*, 1994. *Angew. Chem. Int. Ed. Engl.* 33: 2059; Carell, *et al.*, 1994. *Angew. Chem. Int. Ed. Engl.* 33: 2061; and Gallop, *et al.*, 1994. *J. Med. Chem.* 37: 1233.

Libraries of compounds may be presented in solution (*e.g.*, Houghten, 1992. *Biotechniques* 13: 412-421), or on beads (Lam, 1991. *Nature* 354: 82-84), on chips (Fodor, 1993. *Nature* 364: 555-556), bacteria (Ladner, U.S. Patent No. 5,223,409), spores (Ladner, U.S. Patent 5,233,409), plasmids (Cull, *et al.*, 1992. *Proc. Natl. Acad. Sci. USA* 89: 1865-1869) or on phage (Scott and Smith, 1990. *Science* 249: 386-390; Devlin, 1990. *Science* 249: 404-406; Cwirla, *et al.*, 1990. *Proc. Natl. Acad. Sci. U.S.A.* 87: 6378-6382; Felici, 1991. *J. Mol. Biol.* 222: 301-310; Ladner, U.S. Patent No. 5,233,409.).

In one embodiment, an assay is a cell-based assay in which a cell which expresses a membrane-bound form of GPCRX protein, or a biologically-active portion thereof, on the cell surface is contacted with a test compound and the ability of the test compound to bind to an GPCRX protein determined. The cell, for example, can of mammalian origin or a yeast cell. Determining the ability of the test compound to bind to the GPCRX protein can be accomplished, for example, by coupling the test compound with a radioisotope or enzymatic label such that binding of the test compound to the GPCRX protein or biologically-active portion thereof can be determined by detecting the labeled compound in a complex. For example, test compounds can be labeled with ^{125}I, ^{35}S, ^{14}C, or ^{3}H, either directly or indirectly, and the radioisotope detected by direct counting of radioemission or by scintillation counting. Alternatively, test compounds can be enzymatically-labeled with, for example, horseradish peroxidase, alkaline phosphatase, or luciferase, and the enzymatic label detected by determination of conversion of an appropriate substrate to product. In one embodiment, the assay comprises contacting a cell which expresses a membrane-bound form of GPCRX protein, or a biologically-active portion thereof, on the cell surface with a known compound which binds GPCRX to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with an GPCRX protein, wherein determining the ability of the test compound to interact with an GPCRX protein comprises determining the ability of the test compound to preferentially bind to GPCRX protein or a biologically-active portion thereof as compared to the known compound.

In another embodiment, an assay is a cell-based assay comprising contacting a cell expressing a membrane-bound form of GPCRX protein, or a biologically-active portion thereof, on the cell surface with a test compound and determining the ability of the test compound to modulate (*e.g.*, stimulate or inhibit) the activity of the GPCRX protein or

biologically-active portion thereof. Determining the ability of the test compound to modulate the activity of GPCRX or a biologically-active portion thereof can be accomplished, for example, by determining the ability of the GPCRX protein to bind to or interact with an GPCRX target molecule. As used herein, a "target molecule" is a molecule with which an GPCRX protein binds or interacts in nature, for example, a molecule on the surface of a cell which expresses an GPCRX interacting protein, a molecule on the surface of a second cell, a molecule in the extracellular milieu, a molecule associated with the internal surface of a cell membrane or a cytoplasmic molecule. An GPCRX target molecule can be a non-GPCRX molecule or an GPCRX protein or polypeptide of the invention. In one embodiment, an GPCRX target molecule is a component of a signal transduction pathway that facilitates transduction of an extracellular signal (*e.g.* a signal generated by binding of a compound to a membrane-bound GPCRX molecule) through the cell membrane and into the cell. The target, for example, can be a second intercellular protein that has catalytic activity or a protein that facilitates the association of downstream signaling molecules with GPCRX.

Determining the ability of the GPCRX protein to bind to or interact with an GPCRX target molecule can be accomplished by one of the methods described above for determining direct binding. In one embodiment, determining the ability of the GPCRX protein to bind to or interact with an GPCRX target molecule can be accomplished by determining the activity of the target molecule. For example, the activity of the target molecule can be determined by detecting induction of a cellular second messenger of the target (*i.e.* intracellular Ca^{2+}, diacylglycerol, IP_3, etc.), detecting catalytic/enzymatic activity of the target an appropriate substrate, detecting the induction of a reporter gene (comprising an GPCRX-responsive regulatory element operatively linked to a nucleic acid encoding a detectable marker, *e.g.*, luciferase), or detecting a cellular response, for example, cell survival, cellular differentiation, or cell proliferation.

In yet another embodiment, an assay of the invention is a cell-free assay comprising contacting an GPCRX protein or biologically-active portion thereof with a test compound and determining the ability of the test compound to bind to the GPCRX protein or biologically-active portion thereof. Binding of the test compound to the GPCRX protein can be determined either directly or indirectly as described above. In one such embodiment, the assay comprises contacting the GPCRX protein or biologically-active portion thereof with a known compound which binds GPCRX to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with an GPCRX protein, wherein determining the ability of the test compound to interact with an GPCRX

protein comprises determining the ability of the test compound to preferentially bind to GPCRX or biologically-active portion thereof as compared to the known compound.

In still another embodiment, an assay is a cell-free assay comprising contacting GPCRX protein or biologically-active portion thereof with a test compound and determining the ability of the test compound to modulate (*e.g.* stimulate or inhibit) the activity of the GPCRX protein or biologically-active portion thereof. Determining the ability of the test compound to modulate the activity of GPCRX can be accomplished, for example, by determining the ability of the GPCRX protein to bind to an GPCRX target molecule by one of the methods described above for determining direct binding. In an alternative embodiment, determining the ability of the test compound to modulate the activity of GPCRX protein can be accomplished by determining the ability of the GPCRX protein further modulate an GPCRX target molecule. For example, the catalytic/enzymatic activity of the target molecule on an appropriate substrate can be determined as described, *supra*.

In yet another embodiment, the cell-free assay comprises contacting the GPCRX protein or biologically-active portion thereof with a known compound which binds GPCRX protein to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with an GPCRX protein, wherein determining the ability of the test compound to interact with an GPCRX protein comprises determining the ability of the GPCRX protein to preferentially bind to or modulate the activity of an GPCRX target molecule.

The cell-free assays of the invention are amenable to use of both the soluble form or the membrane-bound form of GPCRX protein. In the case of cell-free assays comprising the membrane-bound form of GPCRX protein, it may be desirable to utilize a solubilizing agent such that the membrane-bound form of GPCRX protein is maintained in solution. Examples of such solubilizing agents include non-ionic detergents such as n-octylglucoside, n-dodecylglucoside, n-dodecylmaltoside, octanoyl-N-methylglucamide, decanoyl-N-methylglucamide, Triton® X-100, Triton® X-114, Thesit®, Isotridecypoly(ethylene glycol ether)$_n$, N-dodecyl--N,N-dimethyl-3-ammonio-1-propane sulfonate, 3-(3-cholamidopropyl) dimethylamminiol-1-propane sulfonate (CHAPS), or 3-(3-cholamidopropyl)dimethylamminiol-2-hydroxy-1-propane sulfonate (CHAPSO).

In more than one embodiment of the above assay methods of the invention, it may be desirable to immobilize either GPCRX protein or its target molecule to facilitate separation of complexed from uncomplexed forms of one or both of the proteins, as well as to accommodate automation of the assay. Binding of a test compound to GPCRX protein, or interaction of

GPCRX protein with a target molecule in the presence and absence of a candidate compound, can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtiter plates, test tubes, and micro-centrifuge tubes. In one embodiment, a fusion protein can be provided that adds a domain that allows one or both of the proteins to be bound to a matrix. For example, GST-GPCRX fusion proteins or GST-target fusion proteins can be adsorbed onto glutathione sepharose beads (Sigma Chemical, St. Louis, MO) or glutathione derivatized microtiter plates, that are then combined with the test compound or the test compound and either the non-adsorbed target protein or GPCRX protein, and the mixture is incubated under conditions conducive to complex formation (*e.g.*, at physiological conditions for salt and pH). Following incubation, the beads or microtiter plate wells are washed to remove any unbound components, the matrix immobilized in the case of beads, complex determined either directly or indirectly, for example, as described, *supra*. Alternatively, the complexes can be dissociated from the matrix, and the level of GPCRX protein binding or activity determined using standard techniques.

Other techniques for immobilizing proteins on matrices can also be used in the screening assays of the invention. For example, either the GPCRX protein or its target molecule can be immobilized utilizing conjugation of biotin and streptavidin. Biotinylated GPCRX protein or target molecules can be prepared from biotin-NHS (N-hydroxy-succinimide) using techniques well-known within the art (*e.g.*, biotinylation kit, Pierce Chemicals, Rockford, Ill.), and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical). Alternatively, antibodies reactive with GPCRX protein or target molecules, but which do not interfere with binding of the GPCRX protein to its target molecule, can be derivatized to the wells of the plate, and unbound target or GPCRX protein trapped in the wells by antibody conjugation. Methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using antibodies reactive with the GPCRX protein or target molecule, as well as enzyme-linked assays that rely on detecting an enzymatic activity associated with the GPCRX protein or target molecule.

In another embodiment, modulators of GPCRX protein expression are identified in a method wherein a cell is contacted with a candidate compound and the expression of GPCRX mRNA or protein in the cell is determined. The level of expression of GPCRX mRNA or protein in the presence of the candidate compound is compared to the level of expression of GPCRX mRNA or protein in the absence of the candidate compound. The candidate compound can then be identified as a modulator of GPCRX mRNA or protein expression

based upon this comparison. For example, when expression of GPCRX mRNA or protein is greater (*i.e.*, statistically significantly greater) in the presence of the candidate compound than in its absence, the candidate compound is identified as a stimulator of GPCRX mRNA or protein expression. Alternatively, when expression of GPCRX mRNA or protein is less (statistically significantly less) in the presence of the candidate compound than in its absence, the candidate compound is identified as an inhibitor of GPCRX mRNA or protein expression. The level of GPCRX mRNA or protein expression in the cells can be determined by methods described herein for detecting GPCRX mRNA or protein.

In yet another aspect of the invention, the GPCRX proteins can be used as "bait proteins" in a two-hybrid assay or three hybrid assay (*see, e.g.*, U.S. Patent No. 5,283,317; Zervos, *et al.*, 1993. *Cell* 72: 223-232; Madura, *et al.*, 1993. *J. Biol. Chem.* 268: 12046-12054; Bartel, *et al.*, 1993. *Biotechniques* 14: 920-924; Iwabuchi, *et al.*, 1993. *Oncogene* 8: 1693-1696; and Brent WO 94/10300), to identify other proteins that bind to or interact with GPCRX ("GPCRX-binding proteins" or "GPCRX-bp") and modulate GPCRX activity. Such GPCRX-binding proteins are also likely to be involved in the propagation of signals by the GPCRX proteins as, for example, upstream or downstream elements of the GPCRX pathway.

The two-hybrid system is based on the modular nature of most transcription factors, which consist of separable DNA-binding and activation domains. Briefly, the assay utilizes two different DNA constructs. In one construct, the gene that codes for GPCRX is fused to a gene encoding the DNA binding domain of a known transcription factor (*e.g.*, GAL-4). In the other construct, a DNA sequence, from a library of DNA sequences, that encodes an unidentified protein ("prey" or "sample") is fused to a gene that codes for the activation domain of the known transcription factor. If the "bait" and the "prey" proteins are able to interact, *in vivo*, forming an GPCRX-dependent complex, the DNA-binding and activation domains of the transcription factor are brought into close proximity. This proximity allows transcription of a reporter gene (*e.g.*, LacZ) that is operably linked to a transcriptional regulatory site responsive to the transcription factor. Expression of the reporter gene can be detected and cell colonies containing the functional transcription factor can be isolated and used to obtain the cloned gene that encodes the protein which interacts with GPCRX.

The invention further pertains to novel agents identified by the aforementioned screening assays and uses thereof for treatments as described herein.

Detection Assays

Portions or fragments of the cDNA sequences identified herein (and the corresponding complete gene sequences) can be used in numerous ways as polynucleotide reagents. By way of example, and not of limitation, these sequences can be used to: (*i*) map their respective genes on a chromosome; and, thus, locate gene regions associated with genetic disease; (*ii*) identify an individual from a minute biological sample (tissue typing); and (*iii*) aid in forensic identification of a biological sample. Some of these applications are described in the subsections, below.

Chromosome Mapping

Once the sequence (or a portion of the sequence) of a gene has been isolated, this sequence can be used to map the location of the gene on a chromosome. This process is called chromosome mapping. Accordingly, portions or fragments of the GPCRX sequences, SEQ ID NOS:1, 3, 5, 6, 8, 9, 10, 12, 14 and 16, or fragments or derivatives thereof, can be used to map the location of the GPCRX genes, respectively, on a chromosome. The mapping of the GPCRX sequences to chromosomes is an important first step in correlating these sequences with genes associated with disease.

Briefly, GPCRX genes can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp in length) from the GPCRX sequences. Computer analysis of the GPCRX, sequences can be used to rapidly select primers that do not span more than one exon in the genomic DNA, thus complicating the amplification process. These primers can then be used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human gene corresponding to the GPCRX sequences will yield an amplified fragment.

Somatic cell hybrids are prepared by fusing somatic cells from different mammals (*e.g.*, human and mouse cells). As hybrids of human and mouse cells grow and divide, they gradually lose human chromosomes in random order, but retain the mouse chromosomes. By using media in which mouse cells cannot grow, because they lack a particular enzyme, but in which human cells can, the one human chromosome that contains the gene encoding the needed enzyme will be retained. By using various media, panels of hybrid cell lines can be established. Each cell line in a panel contains either a single human chromosome or a small number of human chromosomes, and a full set of mouse chromosomes, allowing easy mapping of individual genes to specific human chromosomes. *See, e.g.,* D'Eustachio, *et al.*, 1983. *Science* 220: 919-924. Somatic cell hybrids containing only fragments of human

chromosomes can also be produced by using human chromosomes with translocations and deletions.

PCR mapping of somatic cell hybrids is a rapid procedure for assigning a particular sequence to a particular chromosome. Three or more sequences can be assigned per day using a single thermal cycler. Using the GPCRX sequences to design oligonucleotide primers, sub-localization can be achieved with panels of fragments from specific chromosomes.

Fluorescence *in situ* hybridization (FISH) of a DNA sequence to a metaphase chromosomal spread can further be used to provide a precise chromosomal location in one step. Chromosome spreads can be made using cells whose division has been blocked in metaphase by a chemical like colcemid that disrupts the mitotic spindle. The chromosomes can be treated briefly with trypsin, and then stained with Giemsa. A pattern of light and dark bands develops on each chromosome, so that the chromosomes can be identified individually. The FISH technique can be used with a DNA sequence as short as 500 or 600 bases. However, clones larger than 1,000 bases have a higher likelihood of binding to a unique chromosomal location with sufficient signal intensity for simple detection. Preferably 1,000 bases, and more preferably 2,000 bases, will suffice to get good results at a reasonable amount of time. For a review of this technique, *see,* Verma, *et al.*, HUMAN CHROMOSOMES: A MANUAL OF BASIC TECHNIQUES (Pergamon Press, New York 1988).

Reagents for chromosome mapping can be used individually to mark a single chromosome or a single site on that chromosome, or panels of reagents can be used for marking multiple sites and/or multiple chromosomes. Reagents corresponding to noncoding regions of the genes actually are preferred for mapping purposes. Coding sequences are more likely to be conserved within gene families, thus increasing the chance of cross hybridizations during chromosomal mapping.

Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. Such data are found, *e.g.*, in McKusick, MENDELIAN INHERITANCE IN MAN, available on-line through Johns Hopkins University Welch Medical Library). The relationship between genes and disease, mapped to the same chromosomal region, can then be identified through linkage analysis (co-inheritance of physically adjacent genes), described in, *e.g.*, Egeland, *et al.*, 1987. *Nature*, 325: 783-787.

Moreover, differences in the DNA sequences between individuals affected and unaffected with a disease associated with the GPCRX gene, can be determined. If a mutation is observed in some or all of the affected individuals but not in any unaffected individuals,

then the mutation is likely to be the causative agent of the particular disease. Comparison of affected and unaffected individuals generally involves first looking for structural alterations in the chromosomes, such as deletions or translocations that are visible from chromosome spreads or detectable using PCR based on that DNA sequence. Ultimately, complete sequencing of genes from several individuals can be performed to confirm the presence of a mutation and to distinguish mutations from polymorphisms.

Tissue Typing

The GPCRX sequences of the invention can also be used to identify individuals from minute biological samples. In this technique, an individual's genomic DNA is digested with one or more restriction enzymes, and probed on a Southern blot to yield unique bands for identification. The sequences of the invention are useful as additional DNA markers for RFLP ("restriction fragment length polymorphisms," described in U.S. Patent No. 5,272,057).

Furthermore, the sequences of the invention can be used to provide an alternative technique that determines the actual base-by-base DNA sequence of selected portions of an individual's genome. Thus, the GPCRX sequences described herein can be used to prepare two PCR primers from the 5'- and 3'-termini of the sequences. These primers can then be used to amplify an individual's DNA and subsequently sequence it.

Panels of corresponding DNA sequences from individuals, prepared in this manner, can provide unique individual identifications, as each individual will have a unique set of such DNA sequences due to allelic differences. The sequences of the invention can be used to obtain such identification sequences from individuals and from tissue. The GPCRX sequences of the invention uniquely represent portions of the human genome. Allelic variation occurs to some degree in the coding regions of these sequences, and to a greater degree in the noncoding regions. It is estimated that allelic variation between individual humans occurs with a frequency of about once per each 500 bases. Much of the allelic variation is due to single nucleotide polymorphisms (SNPs), which include restriction fragment length polymorphisms (RFLPs).

Each of the sequences described herein can, to some degree, be used as a standard against which DNA from an individual can be compared for identification purposes. Because greater numbers of polymorphisms occur in the noncoding regions, fewer sequences are necessary to differentiate individuals. The noncoding sequences can comfortably provide positive individual identification with a panel of perhaps 10 to 1,000 primers that each yield a noncoding amplified sequence of 100 bases. If predicted coding sequences, such as those in

SEQ ID NOS:1, 3, 5, 6, 8, 9, 10, 12, 14 and 16 are used, a more appropriate number of primers for positive individual identification would be 500-2,000.

Predictive Medicine

The invention also pertains to the field of predictive medicine in which diagnostic assays, prognostic assays, pharmacogenomics, and monitoring clinical trials are used for prognostic (predictive) purposes to thereby treat an individual prophylactically. Accordingly, one aspect of the invention relates to diagnostic assays for determining GPCRX protein and/or nucleic acid expression as well as GPCRX activity, in the context of a biological sample (*e.g.*, blood, serum, cells, tissue) to thereby determine whether an individual is afflicted with a disease or disorder, or is at risk of developing a disorder, associated with aberrant GPCRX expression or activity. The disorders include metabolic disorders, diabetes, obesity, infectious disease, anorexia, cancer-associated cachexia, cancer, neurodegenerative disorders, Alzheimer's Disease, Parkinson's Disorder, immune disorders, and hematopoietic disorders, and the various dyslipidemias, metabolic disturbances associated with obesity, the metabolic syndrome X and wasting disorders associated with chronic diseases and various cancers. The invention also provides for prognostic (or predictive) assays for determining whether an individual is at risk of developing a disorder associated with GPCRX protein, nucleic acid expression or activity. For example, mutations in an GPCRX gene can be assayed in a biological sample. Such assays can be used for prognostic or predictive purpose to thereby prophylactically treat an individual prior to the onset of a disorder characterized by or associated with GPCRX protein, nucleic acid expression, or biological activity.

Another aspect of the invention provides methods for determining GPCRX protein, nucleic acid expression or activity in an individual to thereby select appropriate therapeutic or prophylactic agents for that individual (referred to herein as "pharmacogenomics"). Pharmacogenomics allows for the selection of agents (*e.g.*, drugs) for therapeutic or prophylactic treatment of an individual based on the genotype of the individual (*e.g.*, the genotype of the individual examined to determine the ability of the individual to respond to a particular agent.)

Yet another aspect of the invention pertains to monitoring the influence of agents (*e.g.*, drugs, compounds) on the expression or activity of GPCRX in clinical trials.

These and other agents are described in further detail in the following sections.

Diagnostic Assays

An exemplary method for detecting the presence or absence of GPCRX in a biological sample involves obtaining a biological sample from a test subject and contacting the biological sample with a compound or an agent capable of detecting GPCRX protein or nucleic acid (*e.g.*, mRNA, genomic DNA) that encodes GPCRX protein such that the presence of GPCRX is detected in the biological sample. An agent for detecting GPCRX mRNA or genomic DNA is a labeled nucleic acid probe capable of hybridizing to GPCRX mRNA or genomic DNA. The nucleic acid probe can be, for example, a full-length GPCRX nucleic acid, such as the nucleic acid of SEQ ID NOS:1, 3, 5, 6, 8, 9, 10, 12, 14 and 16, or a portion thereof, such as an oligonucleotide of at least 15, 30, 50, 100, 250 or 500 nucleotides in length and sufficient to specifically hybridize under stringent conditions to GPCRX mRNA or genomic DNA. Other suitable probes for use in the diagnostic assays of the invention are described herein.

An agent for detecting GPCRX protein is an antibody capable of binding to GPCRX protein, preferably an antibody with a detectable label. Antibodies can be polyclonal, or more preferably, monoclonal. An intact antibody, or a fragment thereof (*e.g.*, Fab or $F(ab')_2$) can be used. The term "labeled", with regard to the probe or antibody, is intended to encompass direct labeling of the probe or antibody by coupling (*i.e.*, physically linking) a detectable substance to the probe or antibody, as well as indirect labeling of the probe or antibody by reactivity with another reagent that is directly labeled. Examples of indirect labeling include detection of a primary antibody using a fluorescently-labeled secondary antibody and end-labeling of a DNA probe with biotin such that it can be detected with fluorescently-labeled streptavidin. The term "biological sample" is intended to include tissues, cells and biological fluids isolated from a subject, as well as tissues, cells and fluids present within a subject. That is, the detection method of the invention can be used to detect GPCRX mRNA, protein, or genomic DNA in a biological sample *in vitro* as well as *in vivo*. For example, *in vitro* techniques for detection of GPCRX mRNA include Northern hybridizations and *in situ* hybridizations. *In vitro* techniques for detection of GPCRX protein include enzyme linked immunosorbent assays (ELISAs), Western blots, immunoprecipitations, and immunofluorescence. *In vitro* techniques for detection of GPCRX genomic DNA include Southern hybridizations. Furthermore, *in vivo* techniques for detection of GPCRX protein include introducing into a subject a labeled anti-GPCRX antibody. For example, the antibody can be labeled with a radioactive marker whose presence and location in a subject can be detected by standard imaging techniques.

In one embodiment, the biological sample contains protein molecules from the test subject. Alternatively, the biological sample can contain mRNA molecules from the test subject or genomic DNA molecules from the test subject. A preferred biological sample is a peripheral blood leukocyte sample isolated by conventional means from a subject.

In another embodiment, the methods further involve obtaining a control biological sample from a control subject, contacting the control sample with a compound or agent capable of detecting GPCRX protein, mRNA, or genomic DNA, such that the presence of GPCRX protein, mRNA or genomic DNA is detected in the biological sample, and comparing the presence of GPCRX protein, mRNA or genomic DNA in the control sample with the presence of GPCRX protein, mRNA or genomic DNA in the test sample.

The invention also encompasses kits for detecting the presence of GPCRX in a biological sample. For example, the kit can comprise: a labeled compound or agent capable of detecting GPCRX protein or mRNA in a biological sample; means for determining the amount of GPCRX in the sample; and means for comparing the amount of GPCRX in the sample with a standard. The compound or agent can be packaged in a suitable container. The kit can further comprise instructions for using the kit to detect GPCRX protein or nucleic acid.

Prognostic Assays

The diagnostic methods described herein can furthermore be utilized to identify subjects having or at risk of developing a disease or disorder associated with aberrant GPCRX expression or activity. For example, the assays described herein, such as the preceding diagnostic assays or the following assays, can be utilized to identify a subject having or at risk of developing a disorder associated with GPCRX protein, nucleic acid expression or activity. Alternatively, the prognostic assays can be utilized to identify a subject having or at risk for developing a disease or disorder. Thus, the invention provides a method for identifying a disease or disorder associated with aberrant GPCRX expression or activity in which a test sample is obtained from a subject and GPCRX protein or nucleic acid (*e.g.*, mRNA, genomic DNA) is detected, wherein the presence of GPCRX protein or nucleic acid is diagnostic for a subject having or at risk of developing a disease or disorder associated with aberrant GPCRX expression or activity. As used herein, a "test sample" refers to a biological sample obtained from a subject of interest. For example, a test sample can be a biological fluid (*e.g.*, serum), cell sample, or tissue.

Furthermore, the prognostic assays described herein can be used to determine whether a subject can be administered an agent (*e.g.*, an agonist, antagonist, peptidomimetic, protein,

peptide, nucleic acid, small molecule, or other drug candidate) to treat a disease or disorder associated with aberrant GPCRX expression or activity. For example, such methods can be used to determine whether a subject can be effectively treated with an agent for a disorder. Thus, the invention provides methods for determining whether a subject can be effectively treated with an agent for a disorder associated with aberrant GPCRX expression or activity in which a test sample is obtained and GPCRX protein or nucleic acid is detected (*e.g.*, wherein the presence of GPCRX protein or nucleic acid is diagnostic for a subject that can be administered the agent to treat a disorder associated with aberrant GPCRX expression or activity).

The methods of the invention can also be used to detect genetic lesions in an GPCRX gene, thereby determining if a subject with the lesioned gene is at risk for a disorder characterized by aberrant cell proliferation and/or differentiation. In various embodiments, the methods include detecting, in a sample of cells from the subject, the presence or absence of a genetic lesion characterized by at least one of an alteration affecting the integrity of a gene encoding an GPCRX-protein, or the misexpression of the GPCRX gene. For example, such genetic lesions can be detected by ascertaining the existence of at least one of: (*i*) a deletion of one or more nucleotides from an GPCRX gene; (*ii*) an addition of one or more nucleotides to an GPCRX gene; (*iii*) a substitution of one or more nucleotides of an GPCRX gene, (*iv*) a chromosomal rearrangement of an GPCRX gene; (*v*) an alteration in the level of a messenger RNA transcript of an GPCRX gene, (*vi*) aberrant modification of an GPCRX gene, such as of the methylation pattern of the genomic DNA, (*vii*) the presence of a non-wild-type splicing pattern of a messenger RNA transcript of an GPCRX gene, (*viii*) a non-wild-type level of an GPCRX protein, (*ix*) allelic loss of an GPCRX gene, and (*x*) inappropriate post-translational modification of an GPCRX protein. As described herein, there are a large number of assay techniques known in the art which can be used for detecting lesions in an GPCRX gene. A preferred biological sample is a peripheral blood leukocyte sample isolated by conventional means from a subject. However, any biological sample containing nucleated cells may be used, including, for example, buccal mucosal cells.

In certain embodiments, detection of the lesion involves the use of a probe/primer in a polymerase chain reaction (PCR) (*see, e.g.*, U.S. Patent Nos. 4,683,195 and 4,683,202), such as anchor PCR or RACE PCR, or, alternatively, in a ligation chain reaction (LCR) (*see, e.g.*, Landegran, *et al.*, 1988. *Science* 241: 1077-1080; and Nakazawa, *et al.*, 1994. *Proc. Natl. Acad. Sci. USA* 91: 360-364), the latter of which can be particularly useful for detecting point mutations in the GPCRX-gene (*see,* Abravaya, *et al.*, 1995. *Nucl. Acids Res.* 23: 675-682).

This method can include the steps of collecting a sample of cells from a patient, isolating nucleic acid (*e.g.*, genomic, mRNA or both) from the cells of the sample, contacting the nucleic acid sample with one or more primers that specifically hybridize to an GPCRX gene under conditions such that hybridization and amplification of the GPCRX gene (if present) occurs, and detecting the presence or absence of an amplification product, or detecting the size of the amplification product and comparing the length to a control sample. It is anticipated that PCR and/or LCR may be desirable to use as a preliminary amplification step in conjunction with any of the techniques used for detecting mutations described herein.

Alternative amplification methods include: self sustained sequence replication (*see,* Guatelli, *et al.*, 1990. *Proc. Natl. Acad. Sci. USA* 87: 1874-1878), transcriptional amplification system (*see,* Kwoh, *et al.*, 1989. *Proc. Natl. Acad. Sci. USA* 86: 1173-1177); Qβ Replicase (*see,* Lizardi, *et al*, 1988. *BioTechnology* 6: 1197), or any other nucleic acid amplification method, followed by the detection of the amplified molecules using techniques well known to those of skill in the art. These detection schemes are especially useful for the detection of nucleic acid molecules if such molecules are present in very low numbers.

In an alternative embodiment, mutations in an GPCRX gene from a sample cell can be identified by alterations in restriction enzyme cleavage patterns. For example, sample and control DNA is isolated, amplified (optionally), digested with one or more restriction endonucleases, and fragment length sizes are determined by gel electrophoresis and compared. Differences in fragment length sizes between sample and control DNA indicates mutations in the sample DNA. Moreover, the use of sequence specific ribozymes (*see, e.g.,* U.S. Patent No. 5,493,531) can be used to score for the presence of specific mutations by development or loss of a ribozyme cleavage site.

In other embodiments, genetic mutations in GPCRX can be identified by hybridizing a sample and control nucleic acids, *e.g.*, DNA or RNA, to high-density arrays containing hundreds or thousands of oligonucleotides probes. *See, e.g.,* Cronin, *et al.*, 1996. *Human Mutation* 7: 244-255; Kozal, *et al.*, 1996. *Nat. Med.* 2: 753-759. For example, genetic mutations in GPCRX can be identified in two dimensional arrays containing light-generated DNA probes as described in Cronin, *et al.*, *supra*. Briefly, a first hybridization array of probes can be used to scan through long stretches of DNA in a sample and control to identify base changes between the sequences by making linear arrays of sequential overlapping probes. This step allows the identification of point mutations. This is followed by a second hybridization array that allows the characterization of specific mutations by using smaller, specialized probe arrays complementary to all variants or mutations detected. Each mutation

array is composed of parallel probe sets, one complementary to the wild-type gene and the other complementary to the mutant gene.

In yet another embodiment, any of a variety of sequencing reactions known in the art can be used to directly sequence the GPCRX gene and detect mutations by comparing the sequence of the sample GPCRX with the corresponding wild-type (control) sequence. Examples of sequencing reactions include those based on techniques developed by Maxim and Gilbert, 1977. *Proc. Natl. Acad. Sci. USA* 74: 560 or Sanger, 1977. *Proc. Natl. Acad. Sci. USA* 74: 5463. It is also contemplated that any of a variety of automated sequencing procedures can be utilized when performing the diagnostic assays (*see, e.g.,* Naeve, *et al.*, 1995. *Biotechniques* 19: 448), including sequencing by mass spectrometry (see, *e.g.*, PCT International Publication No. WO 94/16101; Cohen, *et al.*, 1996. *Adv. Chromatography* 36: 127-162; and Griffin, *et al.*, 1993. *Appl. Biochem. Biotechnol.* 38: 147-159).

Other methods for detecting mutations in the GPCRX gene include methods in which protection from cleavage agents is used to detect mismatched bases in RNA/RNA or RNA/DNA heteroduplexes. *See, e.g.,* Myers, *et al.*, 1985. *Science* 230: 1242. In general, the art technique of "mismatch cleavage" starts by providing heteroduplexes of formed by hybridizing (labeled) RNA or DNA containing the wild-type GPCRX sequence with potentially mutant RNA or DNA obtained from a tissue sample. The double-stranded duplexes are treated with an agent that cleaves single-stranded regions of the duplex such as which will exist due to basepair mismatches between the control and sample strands. For instance, RNA/DNA duplexes can be treated with RNase and DNA/DNA hybrids treated with S_1 nuclease to enzymatically digesting the mismatched regions. In other embodiments, either DNA/DNA or RNA/DNA duplexes can be treated with hydroxylamine or osmium tetroxide and with piperidine in order to digest mismatched regions. After digestion of the mismatched regions, the resulting material is then separated by size on denaturing polyacrylamide gels to determine the site of mutation. *See, e.g.,* Cotton, *et al.*, 1988. *Proc. Natl. Acad. Sci. USA* 85: 4397; Saleeba, *et al.*, 1992. *Methods Enzymol.* 217: 286-295. In an embodiment, the control DNA or RNA can be labeled for detection.

In still another embodiment, the mismatch cleavage reaction employs one or more proteins that recognize mismatched base pairs in double-stranded DNA (so called "DNA mismatch repair" enzymes) in defined systems for detecting and mapping point mutations in GPCRX cDNAs obtained from samples of cells. For example, the mutY enzyme of *E. coli* cleaves A at G/A mismatches and the thymidine DNA glycosylase from HeLa cells cleaves T at G/T mismatches. *See, e.g.,* Hsu, *et al.*, 1994. *Carcinogenesis* 15: 1657-1662. According to

an exemplary embodiment, a probe based on an GPCRX sequence, *e.g.*, a wild-type GPCRX sequence, is hybridized to a cDNA or other DNA product from a test cell(s). The duplex is treated with a DNA mismatch repair enzyme, and the cleavage products, if any, can be detected from electrophoresis protocols or the like. *See, e.g.*, U.S. Patent No. 5,459,039.

In other embodiments, alterations in electrophoretic mobility will be used to identify mutations in GPCRX genes. For example, single strand conformation polymorphism (SSCP) may be used to detect differences in electrophoretic mobility between mutant and wild type nucleic acids. *See, e.g.*, Orita, *et al.*, 1989. *Proc. Natl. Acad. Sci. USA*: 86: 2766; Cotton, 1993. *Mutat. Res.* 285: 125-144; Hayashi, 1992. *Genet. Anal. Tech. Appl.* 9: 73-79.

Single-stranded DNA fragments of sample and control GPCRX nucleic acids will be denatured and allowed to renature. The secondary structure of single-stranded nucleic acids varies according to sequence, the resulting alteration in electrophoretic mobility enables the detection of even a single base change. The DNA fragments may be labeled or detected with labeled probes. The sensitivity of the assay may be enhanced by using RNA (rather than DNA), in which the secondary structure is more sensitive to a change in sequence. In one embodiment, the subject method utilizes heteroduplex analysis to separate double stranded heteroduplex molecules on the basis of changes in electrophoretic mobility. *See, e.g.*, Keen, *et al.*, 1991. *Trends Genet.* 7: 5.

In yet another embodiment, the movement of mutant or wild-type fragments in polyacrylamide gels containing a gradient of denaturant is assayed using denaturing gradient gel electrophoresis (DGGE). *See, e.g.*, Myers, *et al.*, 1985. *Nature* 313: 495. When DGGE is used as the method of analysis, DNA will be modified to insure that it does not completely denature, for example by adding a GC clamp of approximately 40 bp of high-melting GC-rich DNA by PCR. In a further embodiment, a temperature gradient is used in place of a denaturing gradient to identify differences in the mobility of control and sample DNA. *See, e.g.*, Rosenbaum and Reissner, 1987. *Biophys. Chem.* 265: 12753.

Examples of other techniques for detecting point mutations include, but are not limited to, selective oligonucleotide hybridization, selective amplification, or selective primer extension. For example, oligonucleotide primers may be prepared in which the known mutation is placed centrally and then hybridized to target DNA under conditions that permit hybridization only if a perfect match is found. *See, e.g.*, Saiki, *et al.*, 1986. *Nature* 324: 163; Saiki, *et al.*, 1989. *Proc. Natl. Acad. Sci. USA* 86: 6230. Such allele specific oligonucleotides are hybridized to PCR amplified target DNA or a number of different mutations when the

oligonucleotides are attached to the hybridizing membrane and hybridized with labeled target DNA.

Alternatively, allele specific amplification technology that depends on selective PCR amplification may be used in conjunction with the instant invention. Oligonucleotides used as primers for specific amplification may carry the mutation of interest in the center of the molecule (so that amplification depends on differential hybridization; *see, e.g.*, Gibbs, *et al.*, 1989. *Nucl. Acids Res.* 17: 2437-2448) or at the extreme 3'-terminus of one primer where, under appropriate conditions, mismatch can prevent, or reduce polymerase extension (*see, e.g.*, Prossner, 1993. *Tibtech.* 11: 238). In addition it may be desirable to introduce a novel restriction site in the region of the mutation to create cleavage-based detection. *See, e.g.*, Gasparini, *et al.*, 1992. *Mol. Cell Probes* 6: 1. It is anticipated that in certain embodiments amplification may also be performed using *Taq* ligase for amplification. *See, e.g.*, Barany, 1991. *Proc. Natl. Acad. Sci. USA* 88: 189. In such cases, ligation will occur only if there is a perfect match at the 3'-terminus of the 5' sequence, making it possible to detect the presence of a known mutation at a specific site by looking for the presence or absence of amplification.

The methods described herein may be performed, for example, by utilizing pre-packaged diagnostic kits comprising at least one probe nucleic acid or antibody reagent described herein, which may be conveniently used, *e.g.*, in clinical settings to diagnose patients exhibiting symptoms or family history of a disease or illness involving an GPCRX gene.

Furthermore, any cell type or tissue, preferably peripheral blood leukocytes, in which GPCRX is expressed may be utilized in the prognostic assays described herein. However, any biological sample containing nucleated cells may be used, including, for example, buccal mucosal cells.

Pharmacogenomics

Agents, or modulators that have a stimulatory or inhibitory effect on GPCRX activity (*e.g.*, GPCRX gene expression), as identified by a screening assay described herein can be administered to individuals to treat (prophylactically or therapeutically) disorders (The disorders include metabolic disorders, diabetes, obesity, infectious disease, anorexia, cancer-associated cachexia, cancer, neurodegenerative disorders, Alzheimer's Disease, Parkinson's Disorder, immune disorders, and hematopoietic disorders, and the various dyslipidemias, metabolic disturbances associated with obesity, the metabolic syndrome X and wasting disorders associated with chronic diseases and various cancers.) In conjunction with such

treatment, the pharmacogenomics (*i.e.*, the study of the relationship between an individual's genotype and that individual's response to a foreign compound or drug) of the individual may be considered. Differences in metabolism of therapeutics can lead to severe toxicity or therapeutic failure by altering the relation between dose and blood concentration of the pharmacologically active drug. Thus, the pharmacogenomics of the individual permits the selection of effective agents (*e.g.*, drugs) for prophylactic or therapeutic treatments based on a consideration of the individual's genotype. Such pharmacogenomics can further be used to determine appropriate dosages and therapeutic regimens. Accordingly, the activity of GPCRX protein, expression of GPCRX nucleic acid, or mutation content of GPCRX genes in an individual can be determined to thereby select appropriate agent(s) for therapeutic or prophylactic treatment of the individual.

Pharmacogenomics deals with clinically significant hereditary variations in the response to drugs due to altered drug disposition and abnormal action in affected persons. See *e.g.*, Eichelbaum, 1996. *Clin. Exp. Pharmacol. Physiol.*, 23: 983-985; Linder, 1997. *Clin. Chem.*, 43: 254-266. In general, two types of pharmacogenetic conditions can be differentiated. Genetic conditions transmitted as a single factor altering the way drugs act on the body (altered drug action) or genetic conditions transmitted as single factors altering the way the body acts on drugs (altered drug metabolism). These pharmacogenetic conditions can occur either as rare defects or as polymorphisms. For example, glucose-6-phosphate dehydrogenase (G6PD) deficiency is a common inherited enzymopathy in which the main clinical complication is hemolysis after ingestion of oxidant drugs (anti-malarials, sulfonamides, analgesics, nitrofurans) and consumption of fava beans.

As an illustrative embodiment, the activity of drug metabolizing enzymes is a major determinant of both the intensity and duration of drug action. The discovery of genetic polymorphisms of drug metabolizing enzymes (*e.g.*, N-acetyltransferase 2 (NAT 2) and cytochrome P450 enzymes CYP2D6 and CYP2C19) has provided an explanation as to why some patients do not obtain the expected drug effects or show exaggerated drug response and serious toxicity after taking the standard and safe dose of a drug. These polymorphisms are expressed in two phenotypes in the population, the extensive metabolizer (EM) and poor metabolizer (PM). The prevalence of PM is different among different populations. For example, the gene coding for CYP2D6 is highly polymorphic and several mutations have been identified in PM, which all lead to the absence of functional CYP2D6. Poor metabolizers of CYP2D6 and CYP2C19 quite frequently experience exaggerated drug response and side effects when they receive standard doses. If a metabolite is the active therapeutic moiety, PM

show no therapeutic response, as demonstrated for the analgesic effect of codeine mediated by its CYP2D6-formed metabolite morphine. At the other extreme are the so called ultra-rapid metabolizers who do not respond to standard doses. Recently, the molecular basis of ultra-rapid metabolism has been identified to be due to CYP2D6 gene amplification.

Thus, the activity of GPCRX protein, expression of GPCRX nucleic acid, or mutation content of GPCRX genes in an individual can be determined to thereby select appropriate agent(s) for therapeutic or prophylactic treatment of the individual. In addition, pharmacogenetic studies can be used to apply genotyping of polymorphic alleles encoding drug-metabolizing enzymes to the identification of an individual's drug responsiveness phenotype. This knowledge, when applied to dosing or drug selection, can avoid adverse reactions or therapeutic failure and thus enhance therapeutic or prophylactic efficiency when treating a subject with an GPCRX modulator, such as a modulator identified by one of the exemplary screening assays described herein.

Monitoring of Effects During Clinical Trials

Monitoring the influence of agents (*e.g.*, drugs, compounds) on the expression or activity of GPCRX (*e.g.*, the ability to modulate aberrant cell proliferation and/or differentiation) can be applied not only in basic drug screening, but also in clinical trials. For example, the effectiveness of an agent determined by a screening assay as described herein to increase GPCRX gene expression, protein levels, or upregulate GPCRX activity, can be monitored in clinical trails of subjects exhibiting decreased GPCRX gene expression, protein levels, or downregulated GPCRX activity. Alternatively, the effectiveness of an agent determined by a screening assay to decrease GPCRX gene expression, protein levels, or downregulate GPCRX activity, can be monitored in clinical trails of subjects exhibiting increased GPCRX gene expression, protein levels, or upregulated GPCRX activity. In such clinical trials, the expression or activity of GPCRX and, preferably, other genes that have been implicated in, for example, a cellular proliferation or immune disorder can be used as a "read out" or markers of the immune responsiveness of a particular cell.

By way of example, and not of limitation, genes, including GPCRX, that are modulated in cells by treatment with an agent (*e.g.*, compound, drug or small molecule) that modulates GPCRX activity (*e.g.*, identified in a screening assay as described herein) can be identified. Thus, to study the effect of agents on cellular proliferation disorders, for example, in a clinical trial, cells can be isolated and RNA prepared and analyzed for the levels of expression of GPCRX and other genes implicated in the disorder. The levels of gene

expression (*i.e.*, a gene expression pattern) can be quantified by Northern blot analysis or RT-PCR, as described herein, or alternatively by measuring the amount of protein produced, by one of the methods as described herein, or by measuring the levels of activity of GPCRX or other genes. In this manner, the gene expression pattern can serve as a marker, indicative of the physiological response of the cells to the agent. Accordingly, this response state may be determined before, and at various points during, treatment of the individual with the agent.

In one embodiment, the invention provides a method for monitoring the effectiveness of treatment of a subject with an agent (*e.g.*, an agonist, antagonist, protein, peptide, peptidomimetic, nucleic acid, small molecule, or other drug candidate identified by the screening assays described herein) comprising the steps of (*i*) obtaining a pre-administration sample from a subject prior to administration of the agent; (*ii*) detecting the level of expression of an GPCRX protein, mRNA, or genomic DNA in the preadministration sample; (*iii*) obtaining one or more post-administration samples from the subject; (*iv*) detecting the level of expression or activity of the GPCRX protein, mRNA, or genomic DNA in the post-administration samples; (*v*) comparing the level of expression or activity of the GPCRX protein, mRNA, or genomic DNA in the pre-administration sample with the GPCRX protein, mRNA, or genomic DNA in the post administration sample or samples; and (*vi*) altering the administration of the agent to the subject accordingly. For example, increased administration of the agent may be desirable to increase the expression or activity of GPCRX to higher levels than detected, *i.e.*, to increase the effectiveness of the agent. Alternatively, decreased administration of the agent may be desirable to decrease expression or activity of GPCRX to lower levels than detected, *i.e.*, to decrease the effectiveness of the agent.

Methods of Treatment

The invention provides for both prophylactic and therapeutic methods of treating a subject at risk of (or susceptible to) a disorder or having a disorder associated with aberrant GPCRX expression or activity. The disorders include cardiomyopathy, atherosclerosis, hypertension, congenital heart defects, aortic stenosis, atrial septal defect (ASD), atrioventricular (A-V) canal defect, ductus arteriosus, pulmonary stenosis, subaortic stenosis, ventricular septal defect (VSD), valve diseases, tuberous sclerosis, scleroderma, obesity, transplantation, adrenoleukodystrophy, congenital adrenal hyperplasia, prostate cancer, neoplasm; adenocarcinoma, lymphoma, uterus cancer, fertility, hemophilia, hypercoagulation, idiopathic thrombocytopenic purpura, immunodeficiencies, graft versus host disease, AIDS,

bronchial asthma, Crohn's disease; multiple sclerosis, treatment of Albright Hereditary Ostoeodystrophy, and other diseases, disorders and conditions of the like.

These methods of treatment will be discussed more fully, below.

Disease and Disorders

Diseases and disorders that are characterized by increased (relative to a subject not suffering from the disease or disorder) levels or biological activity may be treated with Therapeutics that antagonize (*i.e.*, reduce or inhibit) activity. Therapeutics that antagonize activity may be administered in a therapeutic or prophylactic manner. Therapeutics that may be utilized include, but are not limited to: (*i*) an aforementioned peptide, or analogs, derivatives, fragments or homologs thereof; (*ii*) antibodies to an aforementioned peptide; (*iii*) nucleic acids encoding an aforementioned peptide; (*iv*) administration of antisense nucleic acid and nucleic acids that are "dysfunctional" (*i.e.*, due to a heterologous insertion within the coding sequences of coding sequences to an aforementioned peptide) that are utilized to "knockout" endoggenous function of an aforementioned peptide by homologous recombination (*see, e.g.*, Capecchi, 1989. *Science* 244: 1288-1292); or (*v*) modulators (*i.e.*, inhibitors, agonists and antagonists, including additional peptide mimetic of the invention or antibodies specific to a peptide of the invention) that alter the interaction between an aforementioned peptide and its binding partner.

Diseases and disorders that are characterized by decreased (relative to a subject not suffering from the disease or disorder) levels or biological activity may be treated with Therapeutics that increase (*i.e.*, are agonists to) activity. Therapeutics that upregulate activity may be administered in a therapeutic or prophylactic manner. Therapeutics that may be utilized include, but are not limited to, an aforementioned peptide, or analogs, derivatives, fragments or homologs thereof; or an agonist that increases bioavailability.

Increased or decreased levels can be readily detected by quantifying peptide and/or RNA, by obtaining a patient tissue sample (*e.g.*, from biopsy tissue) and assaying it *in vitro* for RNA or peptide levels, structure and/or activity of the expressed peptides (or mRNAs of an aforementioned peptide). Methods that are well-known within the art include, but are not limited to, immunoassays (*e.g.*, by Western blot analysis, immunoprecipitation followed by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis, immunocytochemistry, etc.) and/or hybridization assays to detect expression of mRNAs (*e.g.*, Northern assays, dot blots, *in situ* hybridization, and the like).

Prophylactic Methods

In one aspect, the invention provides a method for preventing, in a subject, a disease or condition associated with an aberrant GPCRX expression or activity, by administering to the subject an agent that modulates GPCRX expression or at least one GPCRX activity. Subjects at risk for a disease that is caused or contributed to by aberrant GPCRX expression or activity can be identified by, for example, any or a combination of diagnostic or prognostic assays as described herein. Administration of a prophylactic agent can occur prior to the manifestation of symptoms characteristic of the GPCRX aberrancy, such that a disease or disorder is prevented or, alternatively, delayed in its progression. Depending upon the type of GPCRX aberrancy, for example, an GPCRX agonist or GPCRX antagonist agent can be used for treating the subject. The appropriate agent can be determined based on screening assays described herein. The prophylactic methods of the invention are further discussed in the following subsections.

Therapeutic Methods

Another aspect of the invention pertains to methods of modulating GPCRX expression or activity for therapeutic purposes. The modulatory method of the invention involves contacting a cell with an agent that modulates one or more of the activities of GPCRX protein activity associated with the cell. An agent that modulates GPCRX protein activity can be an agent as described herein, such as a nucleic acid or a protein, a naturally-occurring cognate ligand of an GPCRX protein, a peptide, an GPCRX peptidomimetic, or other small molecule. In one embodiment, the agent stimulates one or more GPCRX protein activity. Examples of such stimulatory agents include active GPCRX protein and a nucleic acid molecule encoding GPCRX that has been introduced into the cell. In another embodiment, the agent inhibits one or more GPCRX protein activity. Examples of such inhibitory agents include antisense GPCRX nucleic acid molecules and anti-GPCRX antibodies. These modulatory methods can be performed *in vitro* (*e.g.*, by culturing the cell with the agent) or, alternatively, *in vivo* (*e.g.*, by administering the agent to a subject). As such, the invention provides methods of treating an individual afflicted with a disease or disorder characterized by aberrant expression or activity of an GPCRX protein or nucleic acid molecule. In one embodiment, the method involves administering an agent (*e.g.*, an agent identified by a screening assay described herein), or combination of agents that modulates (*e.g.*, up-regulates or down-regulates) GPCRX expression or activity. In another embodiment, the method involves administering an

GPCRX protein or nucleic acid molecule as therapy to compensate for reduced or aberrant GPCRX expression or activity.

Stimulation of GPCRX activity is desirable in situations in which GPCRX is abnormally downregulated and/or in which increased GPCRX activity is likely to have a beneficial effect. One example of such a situation is where a subject has a disorder characterized by aberrant cell proliferation and/or differentiation (*e.g.*, cancer or immune associated disorders). Another example of such a situation is where the subject has a gestational disease (*e.g.*, preclampsia).

Determination of the Biological Effect of the Therapeutic

In various embodiments of the invention, suitable *in vitro* or *in vivo* assays are performed to determine the effect of a specific Therapeutic and whether its administration is indicated for treatment of the affected tissue.

In various specific embodiments, *in vitro* assays may be performed with representative cells of the type(s) involved in the patient's disorder, to determine if a given Therapeutic exerts the desired effect upon the cell type(s). Compounds for use in therapy may be tested in suitable animal model systems including, but not limited to rats, mice, chicken, cows, monkeys, rabbits, and the like, prior to testing in human subjects. Similarly, for *in vivo* testing, any of the animal model system known in the art may be used prior to administration to human subjects.

Prophylactic and Therapeutic Uses of the Compositions of the Invention

The GPCRX nucleic acids and proteins of the invention are useful in potential prophylactic and therapeutic applications implicated in a variety of disorders including, but not limited to: metabolic disorders, diabetes, obesity, infectious disease, anorexia, cancer-associated cancer, neurodegenerative disorders, Alzheimer's Disease, Parkinson's Disorder, immune disorders, hematopoietic disorders, and the various dyslipidemias, metabolic disturbances associated with obesity, the metabolic syndrome X and wasting disorders associated with chronic diseases and various cancers.

As an example, a cDNA encoding the GPCRX protein of the invention may be useful in gene therapy, and the protein may be useful when administered to a subject in need thereof. By way of non-limiting example, the compositions of the invention will have efficacy for treatment of patients suffering from: metabolic disorders, diabetes, obesity, infectious disease, anorexia, cancer-associated cachexia, cancer, neurodegenerative disorders, Alzheimer's

Disease, Parkinson's Disorder, immune disorders, hematopoietic disorders, and the various dyslipidemias.

Both the novel nucleic acid encoding the GPCRX protein, and the GPCRX protein of the invention, or fragments thereof, may also be useful in diagnostic applications, wherein the presence or amount of the nucleic acid or the protein are to be assessed. A further use could be as an anti-bacterial molecule (*i.e.*, some peptides have been found to possess anti-bacterial properties). These materials are further useful in the generation of antibodies which immunospecifically-bind to the novel substances of the invention for use in therapeutic or diagnostic methods.

EQUIVALENTS

Although particular embodiments have been disclosed herein in detail, this has been done by way of example for purposes of illustration only, and is not intended to be limiting with respect to the scope of the appended claims, which follow. In particular, it is contemplated by the inventors that various substitutions, alterations, and modifications may be made to the invention without departing from the spirit and scope of the invention as defined by the claims. The choice of nucleic acid starting material, clone of interest, or library type is believed to be a matter of routine for a person of ordinary skill in the art with knowledge of the embodiments described herein. Other aspects, advantages, and modifications considered to be within the scope of the following claims.

WHAT IS CLAIMED IS:

1. An isolated polypeptide comprising an amino acid sequence selected from the group consisting of:

   (a) a mature form of an amino acid sequence selected from the group consisting of SEQ ID NOS:2, 4, 7, 11, 13, 15 and 17;

   (b) a variant of a mature form of an amino acid sequence selected from the group consisting of SEQ ID NOS:2, 4, 7, 11, 13, 15 and 17, wherein one or more amino acid residues in said variant differs from the amino acid sequence of said mature form, provided that said variant differs in no more than 15% of the amino acid residues from the amino acid sequence of said mature form;

   (c) an amino acid sequence selected from the group consisting of SEQ ID NOS:2, 4, 7, 11, 13, 15 and 17; and

   (d) a variant of an amino acid sequence selected from the group consisting of SEQ ID NOS:2, 4, 7, 11, 13, 15 and 17, wherein one or more amino acid residues in said variant differs from the amino acid sequence of said mature form, provided that said variant differs in no more than 15% of amino acid residues from said amino acid sequence.

2 The polypeptide of claim 1, wherein said polypeptide comprises the amino acid sequence of a naturally-occurring allelic variant of an amino acid sequence selected from the group consisting of SEQ ID NOS:2, 4, 7, 11, 13, 15 and 17.

3. The polypeptide of claim 2, wherein said allelic variant comprises an amino acid sequence that is the translation of a nucleic acid sequence differing by a single nucleotide from a nucleic acid sequence selected from the group consisting of SEQ ID NOS:1, 3, 5, 6, 8, 9, 10, 12, 14 and 16.

4. The polypeptide of claim 1, wherein the amino acid sequence of said variant comprises a conservative amino acid substitution.

5. An isolated nucleic acid molecule comprising a nucleic acid sequence encoding a polypeptide comprising an amino acid sequence selected from the group consisting of:

(a) a mature form of an amino acid sequence selected from the group consisting of SEQ ID NOS:2, 4, 7, 11, 13, 15 and 17;

(b) a variant of a mature form of an amino acid sequence selected from the group consisting of SEQ ID NOS:2, 4, 7, 11, 13, 15 and 17, wherein one or more amino acid residues in said variant differs from the amino acid sequence of said mature form, provided that said variant differs in no more than 15% of the amino acid residues from the amino acid sequence of said mature form;

(c) an amino acid sequence selected from the group consisting of SEQ ID NOS:2, 4, 7, 11, 13, 15 and 17;

(d) a variant of an amino acid sequence selected from the group consisting of SEQ ID NOS:2, 4, 7, 11, 13, 15 and 17, wherein one or more amino acid residues in said variant differs from the amino acid sequence of said mature form, provided that said variant differs in no more than 15% of amino acid residues from said amino acid sequence;

(e) a nucleic acid fragment encoding at least a portion of a polypeptide comprising an amino acid sequence chosen from the group consisting of SEQ ID NOS:2, 4, 7, 11, 13, 15 and 17, or a variant of said polypeptide, wherein one or more amino acid residues in said variant differs from the amino acid sequence of said mature form, provided that said variant differs in no more than 15% of amino acid residues from said amino acid sequence; and

(f) a nucleic acid molecule comprising the complement of (a), (b), (c), (d) or (e).

6. The nucleic acid molecule of claim 5, wherein the nucleic acid molecule comprises the nucleotide sequence of a naturally-occurring allelic nucleic acid variant.

7. The nucleic acid molecule of claim 5, wherein the nucleic acid molecule encodes a polypeptide comprising the amino acid sequence of a naturally-occurring polypeptide variant.

8. The nucleic acid molecule of claim 5, wherein the nucleic acid molecule differs by a single nucleotide from a nucleic acid sequence selected from the group consisting of SEQ ID NOS:1, 3, 5, 6, 8, 9, 10, 12, 14, and 16.

9. The nucleic acid molecule of claim 5, wherein said nucleic acid molecule comprises a nucleotide sequence selected from the group consisting of:

(a) a nucleotide sequence selected from the group consisting of SEQ ID NOS:1, 3, 5, 6, 8, 9, 10, 12, 14 and 16;

(b) a nucleotide sequence differing by one or more nucleotides from a nucleotide sequence selected from the group consisting of SEQ ID NOS:1, 3, 5, 6, 8, 9, 10, 12, 14 and 16, provided that no more than 20% of the nucleotides differ from said nucleotide sequence;

(c) a nucleic acid fragment of (a); and

(d) a nucleic acid fragment of (b).

10. The nucleic acid molecule of claim 5, wherein said nucleic acid molecule hybridizes under stringent conditions to a nucleotide sequence chosen from the group consisting of SEQ ID NOS:1, 3, 5, 6, 8, 9, 10, 12, 14 and 16, or a complement of said nucleotide sequence.

11. The nucleic acid molecule of claim 5, wherein the nucleic acid molecule comprises a nucleotide sequence selected from the group consisting of:

(a) a first nucleotide sequence comprising a coding sequence differing by one or more nucleotide sequences from a coding sequence encoding said amino acid sequence, provided that no more than 20% of the nucleotides in the coding sequence in said first nucleotide sequence differ from said coding sequence;

(b) an isolated second polynucleotide that is a complement of the first polynucleotide; and

(c) a nucleic acid fragment of (a) or (b).

12. A vector comprising the nucleic acid molecule of claim 11.

13. The vector of claim 12, further comprising a promoter operably-linked to said nucleic acid molecule.

14. A cell comprising the vector of claim 12.

15. An antibody that binds immunospecifically to the polypeptide of claim 1.

16. The antibody of claim 15, wherein said antibody is a monoclonal antibody.

17. The antibody of claim 15, wherein the antibody is a humanized antibody.

18. A method for determining the presence or amount of the polypeptide of claim 1 in a sample, the method comprising:

(a) providing the sample;

(b) contacting the sample with an antibody that binds immunospecifically to the polypeptide; and

(c) determining the presence or amount of antibody bound to said polypeptide,

thereby determining the presence or amount of polypeptide in said sample.

19. A method for determining the presence or amount of the nucleic acid molecule of claim 5 in a sample, the method comprising:

(a) providing the sample;

(b) contacting the sample with a probe that binds to said nucleic acid molecule; and

(c) determining the presence or amount of the probe bound to said nucleic acid molecule,

thereby determining the presence or amount of the nucleic acid molecule in said sample.

20. The method of claim 19 wherein presence or amount of the nucleic acid molecule is used as a marker for cell or tissue type.

21. The method of claim 20 wherein the cell or tissue type is cancerous.

22. A method of identifying an agent that binds to a polypeptide of claim 1, the method comprising:

(a) contacting said polypeptide with said agent; and

(b) determining whether said agent binds to said polypeptide.

23. The method of claim 22 wherein the agent is a cellular receptor or a downstream effector.

24. A method for identifying an agent that modulates the expression or activity of the polypeptide of claim 1, the method comprising:

(a) providing a cell expressing said polypeptide;

(b) contacting the cell with said agent, and

(c) determining whether the agent modulates expression or activity of said polypeptide,

whereby an alteration in expression or activity of said peptide indicates said agent modulates expression or activity of said polypeptide.

25. A method for modulating the activity of the polypeptide of claim 1, the method comprising contacting a cell sample expressing the polypeptide of said claim with a compound that binds to said polypeptide in an amount sufficient to modulate the activity of the polypeptide.

26. A method of treating or preventing a GPCRX-associated disorder, said method comprising administering to a subject in which such treatment or prevention is desired the polypeptide of claim 1 in an amount sufficient to treat or prevent said GPCRX-associated disorder in said subject.

27. The method of claim 26 wherein the disorder is selected from the group consisting of cardiomyopathy and atherosclerosis.

28. The method of claim 26 wherein the disorder is related to cell signal processing and metabolic pathway modulation.

29. The method of claim 26, wherein said subject is a human.

30. A method of treating or preventing a GPCRX-associated disorder, said method comprising administering to a subject in which such treatment or prevention is desired the nucleic acid of claim 5 in an amount sufficient to treat or prevent said GPCRX-associated disorder in said subject.

31. The method of claim 30 wherein the disorder is selected from the group consisting of cardiomyopathy and atherosclerosis.

32. The method of claim 30 wherein the disorder is related to cell signal processing and metabolic pathway modulation.

33. The method of claim 30, wherein said subject is a human.

34. A method of treating or preventing a GPCRX-associated disorder, said method comprising administering to a subject in which such treatment or prevention is desired the antibody of claim 15 in an amount sufficient to treat or prevent said GPCRX-associated disorder in said subject.

35. The method of claim 34 wherein the disorder is diabetes.

36. The method of claim 34 wherein the disorder is related to cell signal processing and metabolic pathway modulation.

37. The method of claim 34, wherein the subject is a human.

38. A pharmaceutical composition comprising the polypeptide of claim 1 and a pharmaceutically-acceptable carrier.

39. A pharmaceutical composition comprising the nucleic acid molecule of claim 5 and a pharmaceutically-acceptable carrier.

40. A pharmaceutical composition comprising the antibody of claim 15 and a pharmaceutically-acceptable carrier.

41. A kit comprising in one or more containers, the pharmaceutical composition of claim 38.

42. A kit comprising in one or more containers, the pharmaceutical composition of claim 39.

43. A kit comprising in one or more containers, the pharmaceutical composition of claim 40.

44. A method for determining the presence of or predisposition to a disease associated with altered levels of the polypeptide of claim 1 in a first mammalian subject, the method comprising:

(a) measuring the level of expression of the polypeptide in a sample from the first mammalian subject; and

(b) comparing the amount of said polypeptide in the sample of step (a) to the amount of the polypeptide present in a control sample from a second mammalian subject known not to have, or not to be predisposed to, said disease;

wherein an alteration in the expression level of the polypeptide in the first subject as compared to the control sample indicates the presence of or predisposition to said disease.

45. The method of claim 44 wherein the predisposition is to cancers.

46. A method for determining the presence of or predisposition to a disease associated with altered levels of the nucleic acid molecule of claim 5 in a first mammalian subject, the method comprising:

(a) measuring the amount of the nucleic acid in a sample from the first mammalian subject; and

(b) comparing the amount of said nucleic acid in the sample of step (a) to the amount of the nucleic acid present in a control sample from a second mammalian subject known not to have or not be predisposed to, the disease;

wherein an alteration in the level of the nucleic acid in the first subject as compared to the control sample indicates the presence of or predisposition to the disease.

47. The method of claim 46 wherein the predisposition is to a cancer.

48. A method of treating a pathological state in a mammal, the method comprising administering to the mammal a polypeptide in an amount that is sufficient to alleviate the pathological state, wherein the polypeptide is a polypeptide having an amino acid sequence at least 95% identical to a polypeptide comprising an amino acid sequence of at least one of SEQ ID NOS:2, 4, 7, 11, 13, 15, and 17, or a biologically active fragment thereof.

49. A method of treating a pathological state in a mammal, the method comprising administering to the mammal the antibody of claim 15 in an amount sufficient to alleviate the pathological state.